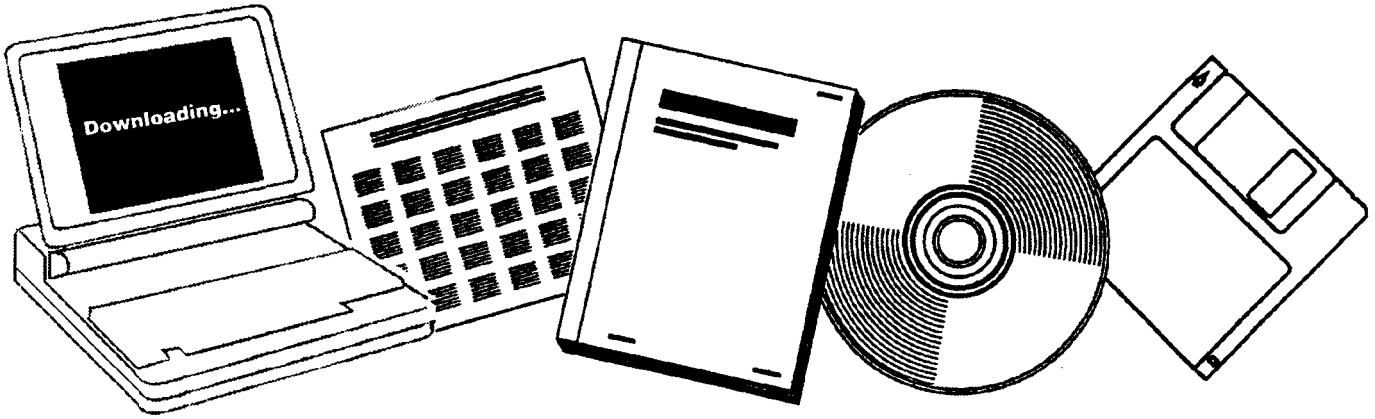
PROCEEDINGS OF THE 1972 LYNDON B. JOHNSON SPACE CENTER ENDOCRINE PROGRAM CONFERENCE

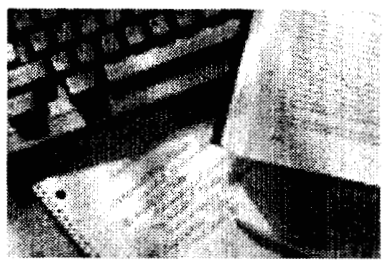
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION. LYNDON B. JOHNSON SPACE CENTER, HOUSTON, TEX

SEP 1974

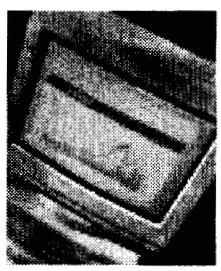


One Source. One Search. One Solution.

NTIS



Providing Permanent, Easy Access to U.S. Government Information



National Technical Information Service is the nation's largest repository and disseminator of government-initiated scientific, technical, engineering, and related business information. The NTIS collection includes almost 3,000,000 information products in a variety of formats: electronic download, online access, CD-ROM, magnetic tape, diskette, multimedia, microfiche and paper.

Search the NTIS Database from 1990 forward

NTIS has upgraded its bibliographic database system and has made all entries since 1990 searchable on **www.ntis.gov.** You now have access to information on more than 600,000 government research information products from this web site.

Link to Full Text Documents at Government Web Sites

Because many Government agencies have their most recent reports available on their own web site, we have added links directly to these reports. When available, you will see a link on the right side of the bibliographic screen.

Download Publications (1997 - Present)

NTIS can now provides the full text of reports as downloadable PDF files. This means that when an agency stops maintaining a report on the web, NTIS will offer a downloadable version. There is a nominal fee for each download for most publications.

For more information visit our website:

www.ntis.gov

U.S. DEPARTMENT OF COMMERCE
Technology Administration
National Technical Information Service
Springfield, VA 22161

JSC-09046

NASA TECHNICAL MEMORANDUM

NASA TM X-58134
September 1974

PROCEEDINGS OF THE 1972

LYNDON B. JOHNSON SPACE CENTER

ENDOCRINE PROGRAM CONFERENCE

Prepared by
Endocrine Laboratory
Life Sciences Directorate

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

LYNDON B. JOHNSON SPACE CENTER

HOUSTON, TEXAS 77058

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
TM X-58134		

4. Title and Subtitle	5. Report Date
PROCEEDINGS OF THE 1972 LYNDON B. JOHNSON SPACE CENTER ENDOCRINE PROGRAM CONFERENCE	September 1974
	6. Performing Organization Code
7. Author(s)	8. Performing Organization Report No.
Carolyn S. Leach, Coordinator	JSC-09046
	10. Work Unit No.
9. Performing Organization Name and Address	951-17-00-00-72
Lyndon B. Johnson Space Center Houston, Texas 77058	11. Contract or Grant No.
	13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address	Technical Memorandum
National Aeronautics and Space Administration Washington, D.C. 20546	14. Sponsoring Agency Code

15. Supplementary Notes

16. Abstract

This document contains the proceedings of the 1972 Lyndon B. Johnson Space Center Endocrine Program Conference. Subjects covered include the following: endocrine/metabolic studies on the Apollo 16 crewmen; changes in glucose, insulin, and growth hormone levels associated with bed rest; circadian rhythms of heart rate and body temperature during 56 days of bed rest; stress-induced changes in corticosteroid metabolism in man; present status of physiological studies on parathyroid hormone and vitamin D; antagonistic effect of lithium on antidiuretic hormone action; proposed Skylab body-fluid volumes study; daily rhythmic changes in serotonin content in areas of the mouse brain and norepinephrine content in areas of the hamster brain; and studies of sodium homeostasis during simulated weightlessness, application of the water immersion model to man.

17. Key Words (Suggested by Author(s))		18. Distribution Statement
*Endocrine Systems	*Apollo 16 Flight	STAR Subject Category: 04.
*Bed Rest	*Skylab Experiment	
*Glucose	*Body Fluids	
*Growth Hormone	*ADH	
*Sodium	*Circadian Rhythms	

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	113	

NASA TM X-58134

PROCEEDINGS OF THE 1972

LYNDON B. JOHNSON SPACE CENTER

ENDOCRINE PROGRAM CONFERENCE

Carolyn S. Leach, Coordinator
Lyndon B. Johnson Space Center
Houston, Texas 77058

INTRODUCTION

By Carolyn S. Leach, Ph. D.

The third Endocrine Program Conference was conducted in November of 1972 at the NASA Lyndon B. Johnson Space Center. The purpose was a part of the continuing effort to fully understand and to evaluate the endocrine changes observed during previous manned missions and, thereby, to further understanding of the physiological adaptation of man to the space-flight environment. Investigators reported on work pertinent to the overall program developed in support of the Apollo missions and the long-duration Skylab flights. The goals of this program continue to be of utmost importance and, indeed, the relevance of these goals has been augmented as man further extends himself and his technology into space. For the purpose of review, these goals are restated here.

1. The establishment (and continuation) of an operational laboratory for immediate endocrinologic assays at the NASA Lyndon B. Johnson Space Center

2. The assembly of a group of endocrine experts who are qualified to advise on procedures and the interpretation of data

3. The advancement of the field of endocrinology by the application of analytical procedures that are low in sample-volume requirements but that are high in specificity

As in previous conferences, the attendees of this third annual NASA Lyndon B. Johnson Space Center Endocrine Program Conference consisted of those who were chosen to help conduct the program. Each participant presented a discussion of work in his area of specialization and related his contributions in support of the program, either with respect to studies or to the development of methodology. The tape-recorded transcripts of these presentations were submitted to the authors for editing and then were compiled into these proceedings.

LIST OF CONTRIBUTORS

1. Carolyn S. Leach, Ph. D.: Head, Endocrine Laboratory, Environmental Physiology Branch, NASA Lyndon B. Johnson Space Center, Houston, Tex.

2. Joan Vernikos-Danellis, Ph. D.: Research Scientist, NASA Ames Research Center, Moffett Field, Calif.

3. Charles M. Winget, Ph. D.: Research Scientist, NASA Ames Research Center, Moffett Field, Calif.

4. Anne L. Goodwin: Research Scientist, NASA Ames Research Center, Moffett Field, Calif.

5. Paul C. Rambaut, Ph. D.: Biomedical Research Division, NASA Lyndon B. Johnson Space Center, Houston, Tex.

6. C. W. DeRoshia: Research Scientist, NASA Ames Research Center, Moffett Field, Calif.

7. S. E. Cronin: Research Scientist, NASA Ames Research Center, Moffett Field, Calif.

8. Martha M. Tacker, Ph. D.: Department of Physiology, Baylor College of Medicine, Houston, Tex.

9. John T. Potts, Jr., M.D.: Chief, Endocrine Unit, Massachusetts General Hospital, Boston, Mass.; Professor of Medicine, Harvard Medical School, Cambridge, Mass.

10. K. G. Swenson: Endocrine Unit, Massachusetts General Hospital, Boston, Mass.

11. Myron Miller, M.D.: Chief, Medical Service, Veterans Administration Hospital; Associate Professor of Medicine, State University of New York, Upstate Medical Center, Syracuse, N.Y.

12. Philip C. Johnson, M.D.: Professor of Medicine and Head of Nuclear Medicine Section, Baylor College of Medicine; Director of Radioisotope Department, Methodist Hospital, Texas Medical Center, Houston, Tex.

13. William W. Morgan, Ph. D.: Department of Anatomy, University of Texas Medical School at San Antonio, San Antonio, Tex.

14. Murray Epstein, M.D.: Assistant Professor of Medicine, University of Miami School of Medicine; Associate Director, Nephrology Section, Veterans Administration Hospital, Miami, Fla.

CONTENTS

1. ENDOCRINE/METABOLIC RESPONSES OF THE APOLLO 16 CREWMEN

By Carolyn S. Leach, Ph. D.

INTRODUCTION

The Endocrine Laboratory conducted biochemical analyses to record the time course, the extent, and the etiology of fluid/electrolyte and hormonal changes resulting from the Apollo 16 crewmen's exposure to space flight. These operational studies were designed to study two aspects of endocrine/metabolic homeostasis. The first group of tests measured fluid/electrolyte concentration changes and examined the mechanisms controlling the changes observed after this and previous missions (refs. 1-1 and 1-2). The radionuclide fluid volume studies were part of this series of tests; they were designed to quantitate the volume changes that accompany prolonged exposure to weightlessness. As in the Apollo 15 mission, total body exchangeable potassium (K) was measured to study further the effect of weightlessness on the metabolism of this intracellular electrolyte.

In the second group of tests, changes in the hormones that are essential for the maintenance of biochemical integrity were recorded. These studies allow an estimate of the physiological cost of space flight to the individual crewman.

METHODS

Approximately 45 milliliters of venous blood were drawn and analyzed 30, 15, and 5 days before the flight (F-30, F-15, and F-5) to ascertain the health status of the crewmen and to establish baseline values for postmission comparison. Similar amounts of blood were drawn as soon as possible (ASAP) after recovery and 1, 3, and 7 days after recovery (R+1, R+3, and R+7). All blood samples, except those drawn ASAP after splashdown, were obtained fasting.

Urine samples were collected from each man every 24 hours beginning the same day as the blood collection. Additional samples were collected during the last 72 hours preflight, the second day during flight, and the first 72 hours postflight. All urine was aliquoted, stabilized, and frozen for future analysis except the in-flight samples, which were preserved with 10 grams of boric acid and held at spacecraft temperatures until after the mission.

The crewmen consumed food of their own choice until 3 days before flight. At that time, a diet of known composition was provided throughout the mission and for 72 hours after recovery.

Analyses of the blood samples (plasma or serum) included, among others: osmolality, sodium (Na), K, chloride (Cl), adrenocorticotrophic hormone (ACTH), angiotensin I, cortisol, human growth hormone (HGH), insulin, and parathyroid hormone. The 24-hour urine samples were analyzed for electrolytes, osmolality, volume, aldosterone, hydrocortisone, antidiuretic hormone (ADH), total and fractionated ketosteroids, and amino acids.

The radionuclide studies were performed on the schedule appearing in table 1-I. Using methods described previously (ref. 1-3), the backup crewmen were studied until F-5; the three control physicians were followed throughout. Calculated radiation exposures for the doses used are shown in table 1-II.

RESULTS

Table 1-III contains the 24-hour urine samples data on the three crewmen. The lunar module pilot's in-flight sample had increased K. Postflight samples from all three crewmen had decreased Na, K, Cl, and volume; osmolality remained unchanged. Titratable acidity did not change from preflight values.

Because of the prolonged exposure to increased temperature, the in-flight urine samples were not suitable for catecholamine or ADH analysis. Epinephrine was elevated during most of the preflight period, and in the postflight period in the lunar module pilot's sample. Norepinephrine remained within normal range throughout the collection periods. Hydrocortisone was elevated in the preflight period for the commander (CDR) and the lunar module pilot (LMP). The in-flight sample demonstrated elevated cortisol for the CDR and the LMP, with normal concentrations in the command module pilot's sample. Total 17-hydroxycorticosteroids were normal to decreased for the in-flight sample and decreased postflight for the CDR and the command module pilot (CMP).

Aldosterone was normal to decreased for the in-flight sample and the immediately postflight sample. Antidiuretic hormone was elevated in the sample from the LMP and the CMP and normal in the CDR's sample immediately postflight. The total 17-ketosteroids demonstrated decreases postflight in all three crewmen and elevated concentration for the in-flight sample on the LMP. The 17-ketosteroid fractionation data given in tables 1-IV(a) to 1-IV(c) demonstrated significant increases in the in-flight sample. For the CDR, these included pregnanediol (PD), androsterone (AND), etiocholanolone (Etio), dehydroepiandrosterone (DHEA), 11=0 etiocholanolone (11=0 Etio), 11-OH androsterone (11-OH AND), and 11-OH etiocholanolone (11-OH Etio); for the LMP, the elevated fractions were PD, AND, Etio, DHEA, 11=0 Etio, 11=0 AND, 11-OH Etio; the CMP had no significant change in the in-flight sample. The postflight samples demonstrated normal patterns (comparable to the preflight patterns) for all three crewmen. However, the R+1 sample of the CMP evidenced a trend toward elevation in PD and 11-OH Etio.

Tables 1-V(a) to 1-V(c) contain the urinary amino acid excretion patterns of all three crewmen. These patterns demonstrate changes in each crewmember for in-flight and postflight samples, and these changes become more

significant considering that from 72 hours preflight they were consuming a monitored dietary intake. Specifically, the glycine excretion of all three men was elevated in the in-flight samples; sarcosine was elevated in the CDR and CMP in the in-flight samples, as were the aminobutyric acids in the CDR and LMP. Postflight glycine was decreased in all crewmen, and taurine was elevated in the CDR and the CMP.

Table 1-VI contains the serum/plasma results. In the ASAP samples, Na and osmolality were slightly elevated and K slightly decreased for all three crewmembers; cortisol decreased slightly for the CDR and the CMP; angiotensin I was markedly elevated for all three men but had returned to normal by the next sample period; and HGH was elevated in the CMP. Insulin and the thyroid hormones demonstrated no changes in any crewmember. Parathyroid hormone did not appear to be significantly changed in the crewmen's samples postflight. All values reported were close to the detection limits of this assay.

The measured body fluid volumes are shown in table 1-VII; these same data, expressed as milliliters per kilogram of body weight, are shown in table 1-VIII. The derived volumes are shown in table 1-IX.

As in other missions, there was a decrease in plasma volume ASAP. The plasma volume deficit had been made up by R+1. Total body water was decreased ASAP, as was the extracellular fluid. The mean percent decrease of intracellular fluid is greater than the percent decrease in the extracellular fluid. As in past missions, the controls showed increased plasma volume and extracellular fluid.

When the same data are corrected for body weight, the plasma volume decreases are reduced to zero. Relative to body weight, there is an increase in total body water, extracellular fluid, and intracellular fluid. Interstitial fluid shows the greatest percent increase.

Table 1-X contains the total body K data. The crewmembers started with higher total body K than did the controls. There was a significant increase in total body exchangeable K of the crew postmission with a tendency to return toward normal at R+7.

DISCUSSION

Results from previous missions have shown that the combined conditions of space flight cause man to react with changes in endocrine/metabolic parameters (ref. 1-4). Although there has been variation in the entent of hormonally induced shifts, fluid/electrolytes have been recorded after every Apollo mission to date (refs. 1-5 and 1-6). In general, the results given in tables 1-II to 1-V do not differ from previously reported data. The exceptions warrant considerable discussion.

Apollo 16 was the first mission since Gemini VII in which urine samples collected during flight from the crewmen were returned for analysis. The

17-hydroxycorticosteroids were found to be significantly decreased during that 14-day Gemini VII mission (ref. 1-7). Likewise, total 17-hydroxycorticosteroids were decreased in the second day in-flight specimen from the Apollo 16 crewmen. Ordinarily, if total 17-OH excretion decreases, a decrease in cortisol would be expected. However, cortisol excretion during this in-flight phase of this mission was significantly elevated in the CDR's and the LMP's specimens and normal in the CMP's specimen. (No value was lower than preflight or postflight values.) The diverging of these results could be related to either a storage program that affected the hydroxycorticosteroid analyses or, more likely, changes in blood flow to the liver that altered the conjugation of the free hormone resulting in decreased excretion of 17-hydroxycorticosteroids.

Postflight decreases in the plasma cortisol and ACTH have been observed after almost all Apollo missions. The exact cause of these decreases remains to be explained (ref. 1-8). However, like the in-flight urine sample, a possibility exists that the decreases measured were related to changes in adrenal steroid conjugation by the liver.

In several endocrine-related diseases, the determination of urinary 17-ketosteroids, either total or fractions, has been helpful in both understanding and diagnosis. In certain other problems as well as in many so-called nonendocrine conditions, the value of the test has been limited because of the normal day-to-day variations in excretion (ref. 1-9). However, in this study, the preflight baseline was sufficient to allow comparisons to be made for each man to his preflight values. In this comparison, certain compounds were significantly changed from preflight values of the CDR and the LMP. These two crewmen had twofold increases in total 17-ketosteroids, which included almost all fractions. The greatest increase was in DHEA. The exact function of this steroid is not known, but its secretion appears to be a response to stress (ref. 1-9). The DHEA has been examined also for its influence on nitrogen and mineral metabolism (ref. 1-10). The fractionated 17-ketosteroid results support other indications of stress in the CDR and the LMP during the flight. Whether the stress was related to their anticipation of the lunar landing or was due to the individual biologic difference from the CMP remains unclear. It is significant to note that the greatest changes were in those compounds of adrenal origin.

The changes in amino acid excretion patterns are thought to be related to diet as well as to muscular activity. However, as in every study of amino acid excretion, renal threshold, glomular filtration rate, and cellular utilization enter into the total explanation (ref. 1-11). Furthermore, the relationship between adrenal steroid activity and amino acid excretion must be considered because it has been established that adrenal steroids alter urinary excretion patterns of amino acids (ref. 1-12). Glycine, significantly elevated in the in-flight sample for all three crewmen, is required by the body for formation of nucleic acid, porphyrins, creatine, hippuric acid, and bile acid conjugates (ref. 1-13). Therefore, the increased excretion of this amino acid could be related to cellular mass loss or to the suspected decrease in liver blood flow. Sarcosine, another amino acid showing elevations during flight, is related to muscle protein and may be indicative of muscle breakdown during flight.

Thyroid activity appears to have undergone no change from preflight to postflight values as measured by thyroid (T) hormones T_3 and T_4. The levels of these circulating hormones give indications of changes in metabolism and in the thyroid gland.

Parathyroid hormone levels were examined on these crewmen to assess calcium and phosphorus metabolism. Because those assessments did not indicate values significantly different from the preflight control values, it is concluded that if changes occurred in calcium metabolism during this mission, they were not reflected by the activity of this hormone.

All three of the crewmen consumed fewer calories during the mission than they did during the preflight control period. Furthermore, data from one day during flight indicate that the crewmen were in negative nitrogen balance. Net protein synthesis in the body is unlikely during decreased food intake. The CMP's postflight increase in HGH may represent an attempt to keep nitrogen loss at a minimum by stimulating protein synthesis to as high a level as possible.

The fluid/electrolyte changes observed during this mission must be viewed in relation to the diet consumed by the crewmen as well as specifics of mission activity. The second day during flight showed no change in urine volume. This result becomes significant when the intake data are reviewed and compared to preflight averages, because there were significant decreases for all three crewmen in water intake on that day. Furthermore, the estimated insensible water loss is high in the command module. Postflight, the 24-hour urine volume was decreased in all three crewmen. This finding agrees with data from previous Apollo missions (except that of Apollo 15). Similarly, the urinary and serum electrolyte results agree with those of other missions. The in-flight urine sample added previously unavailable information on normal electrolyte content except for the increased K in one crewman's urine. Because only one collection day was analyzed, it is not possible to establish whether this was a constant occurrence or one unique to the second day during flight. There were postflight decreases in Na, K, and Cl as well as in volume.

Urinary ADH increased significantly postflight. This finding, which has been constant for all Apollo missions when the measurement was made, is considered to reflect the need to readjust water balance postflight, thereby restoring circulating blood volume to a level more consistent with the return of blood from the lower extremities under the influence of gravity.

Aldosterone was found to be markedly elevated during flight for the Gemini VII crewmen. The urine measured was collected on mission days 5-6, 7-8, 9-10, 11-12, and 13-14 (ref. 1-7). The Apollo 16 results demonstrated decreased to normal aldosterone on the second day of flight. The difference in these results could lie in the time of collection. It is not uncommon for the development of a secondary aldosteronism to take several days after appropriate metabolic stimuli. An alternate explanation could be related to unknown technical differences that are unrelated to the crewmen's responses to the missions.

In all three crewmen, the postflight elevation in renin activity, measured as angiotensin I, supports the previous findings that blood flow through the kidney changes after recovery. It is interesting to note that even with rises in renin activity, there was no increase in aldosterone postflight. The reason for this result is not clear; however, the data must be viewed in terms of the electrolyte intake (ref. 1-14) or it would be assumed that the renin elevation was transitory to postural changes (ref. 1-15).

All Apollo missions, including Apollo 16, have been followed by a change in the plasma volume of the returning crewmen. The overall mean of the crewmen's plasma volume decrease for six Apollo missions (7, 8, 9, 14, 15, and 16) is -5.5 ± 1.4 percent. This decrease is greater than the -2.4 percent found after three Moon landing missions (Apollo 14, 15, and 16). These changes are considerably less than the -10 percent mean loss associated with an equivalent period of bed rest (ref. 1-16). Only 3 of the 18 crewmembers have ever shown decrease greater than the usual 10 percent. A smaller decrease in plasma volume could be one manifestation of an in-flight increase in adrenal activity, particularly aldosterone. A drop of only -2.4 percent in plasma volume would not be expected to produce postmission postural hypotension or sensitivity to lower body negative pressure. Because no plasma volume measurements have been performed in orbital flight, it is not known whether the plasma volume actually had been lower during the mission and increased slightly to the point at which it was recorded ASAP or whether plasma volume was essentially stable, after the -5.5 percent decrease had occurred. Analysis of the Gemini data suggests that the former explanation is the more likely of the two. The plasma volume changes of the crewmen contrast dramatically with the control subjects' mean plasma volume, which was 14.3 percent or, corrected for body weight, 8.9 percent. The increased plasma volumes of the control subjects probably represent one of their adaptations to the warm to hot environment found on a Navy carrier plying tropical waters.

When the mean values were obtained from the crewmembers of the Apollo 14, 15, and 16 missions, all fluid spaces were decreased ASAP. However, when these spaces were corrected for weight changes, all but the intracellular space was increased. Particularly noticeable is the increase in extracellular space and the interstitial (extravascular) portion of the extracellular fluid volume.

Even with adequate calories available, most crewmen have shown a weight loss postmission. Part of this weight loss is made up during the first 24 hours after recovery. The remainder takes longer to make up, and the crewmen do not return to their premission weight for several days to weeks later. This finding suggests that the weight loss during a mission is divided between loss in tissue and loss in fluid. Fluid loss could be made up within the first 24 hours, whereas recovery of tissue losses would take considerably longer.

Weight loss from short-term dieting is generally followed by an increase in extracellular fluid that compensates for the tissues lost. This extra fluid is ordinarily lost by diuresis at irregular intervals of several days to several weeks. The increased extracellular fluid volume seen after these missions could be explained as a compensation for tissue losses. The water retention associated with weight loss is probably accomplished by increased

aldosterone secretion. Increased aldosterone secretion has been found during the Gemini VII flight; and the ASAP urine samples, plasma electrolyte, and total body K changes are compatible with in-flight increases of aldosterone secretion.

The determinations of total exchangeable K were internally consistent as shown by the reproducibility of the control subject values. The results from the flight crew are quite different and showed an increase in exchangeable K on F-5 and ASAP when determined as milliequivalents per kilogram of body weight. However, an increase is seen in only one crewmember in the total milliequivalents exchangeable K in the body ASAP. A change in body weight unrelated to K-containing spaces would affect the values expressed on the basis of milliliters per kilogram of body weight. An increase in exchangeable K could represent a true increase in metabolically active K (e.g., muscle), could represent increased unabsorbed K in the gastrointestinal tract, or could indicate that the body contains multiple K spaces and that conditions were right ASAP to measure a space which ordinarily mixes poorly with the radioactivity in the 24 to 48 hours used for determination of the equilibrium space. Poor absorption is probably ruled out because fecal specimens did not show excessive radioactive K loss. Studies of the red cells showed decreased red-cell K with decreased adenosine triphosphate; this relationship suggests that permeability to K was increased. Because the red-cell K ordinarily equilibrates poorly in 48 hours, a leak of K would be expected to have produced a larger total body space. However, the red-cell K is not great enough to account for the results obtained. Therefore, it would have to be assumed that other tissues shared the increased K turnover of the red cells.

REFERENCES

1-1. Leach, C. S.; Alexander, W. C.; and Fischer, C. L.: Compensatory Changes During Adaptation to the Weightlessness Environment. The Physiologist, vol. 13, no. 3, 1970, p. 246.

1-2. Leach, Carolyn S.; Alexander, W. Carter; and Johnson, Philip C.: Adrenal and Pituitary Response of the Apollo 15 Crew Members. J. Clin. Endocrine. Metab., vol. 35, no. 5, 1972, pp. 642-645.

1-3. Johnson, P. C.; Driscoll, T. B.; Alexander, W. C.; and Lambertsen, C. J.: Body Fluid Volume Changes During a 14-Day Continuous Exposure to 5.2% O_2 in N_2 at Pressure Equivalent to 100 FSW (4 ata). Aerospace Med., vol. 44, no. 7, July 1973, pp. 860-863.

1-4. Berry, Charles A.: Space Medicine in Perspective: A Critical Review of the Manned Space Program. JAMA, vol. 201, no. 4, 1967, p. 232.

1-5. Berry, Charles A.: Summary of Medical Experience in the Apollo 7 through 11 Manned Spaceflights. Aerospace Med., vol. 41, no. 5, May 1970, p. 500.

1-6. Leach, C. S.; Alexander, W. C.; and Johnson P. C.: Endocrine/Metabolic Changes in Apollo Astronauts. Fourth International Congress of Endocrinology, June 18-24, 1972 (Excerpta Medica).

1-7. Lutwak, Leo; Whedon, G. Donald; Lachance, Paul A.; Reid, Jeanne M.; and Lipscomb, Harry S.: Mineral, Electrolyte and Nitrogen Balance Studies of the Gemini-VII Fourteen-Day Orbital Space Flight. J. Clin. Endocrin. Metab., vol. 29, no. 9, Sept. 1969, pp. 1140-1156.

1-8. Leach, C. S.; and Campbell, B. O.: Hydrocortisone and ACTH Levels in Manned Spaceflight. Rhythms in Special Environments, Proceedings of the International Society for the Study of Biologic Rhythms, Little Rock Ark., Nov. 1971.

1-9. Keutmann, E. Henry; and Mason, W. B.: Individual Urinary 17-Ketosteroids of Healthy Persons Determined by Gas Chromatography: Biochemical and Clinical Considerations. J. Clin. Endocrin. Metab., vol. 27, no. 3, Mar. 1967, p. 406.

1-10. Lipsett, Mortimer B.; Coffman, Grant D.; and Nixon, Wilbert E.: Lack of Activity of Dehydroepiandrosterone Sulfate in Metabolic Balance Studies. J. Clin. Endocrin. Metab., vol. 25, no. 7, July 1965, pp. 993-994.

1-11. Page, E. W.; Glendering, M. B.; Dignam, W.; and Harper, H. A.: The Causes of Cistidinurea. Am. J. Obstet. Gynec., vol. 68, 1954, p. 110.

1-12. Zinneman, Horace H.; Johnson, Janet J.; and Seal, Ulysses S.: Effect of Short-Term Therapy with Cortisol on the Urinary Excretion of Free Amino Acids. J. Clin. Endocrin. Metab., vol. 23, no. 10, Oct. 1963, pp. 996-1000.

1-13. Searcy, Ronald L.: Diagnostic Biochemistry. McGraw-Hill Book Co., Inc., The Blakiston Division, 1969, p. 50.

1-14. Bailey, Richard E.; and Roberts, Kathleen E.: Increased Aldosterone Excretion During Chronic Sodium Deprivation in Two Male Subjects. Am. J. Med. Sci., vol 239, no. 3, Mar. 1960, pp. 338-340.

1-15. Cohen, E. L.; Rouner, D. P.; Conn, J. W.; and Blough, W. M., Jr.: Use of Peripheral Plasma Renin Activity (PPRA) in the Diagnosis of Renovascular Hypertension. Circ. Res., vol. 13, 1965, p. 424.

1-16. Johnson, Philip C.; Fischer, Craig L.; Leach, Carolyn S.: Hematologic Implications of Hypodynamic States. Sec. 2 of Hypogravic and Hypodynamic Environments. NASA SP-269, 1971.

TABLE 1-I.- SCHEDULE OF RADIONUCLIDE STUDIES

Sample schedule	Analysis date, 1972			Radionuclide volumes			
	Crew	Backup	Control	^{42}K	Extra-cellular fluid	Total body water	Plasma
F-30	Mar. 17	Mar. 17	Mar. 17	X	--	--	--
F-15	Apr. 1	Mar. 31	Mar. 30	X	X	X	X
F-5	Apr. 10	Apr. 10	Apr. 10	X	--	--	--
ASAP	Apr. 26	--	Apr. 25	X	X	X	X
R+1	Apr. 27	--	Apr. 26	X	X	X	X
R+7	May 4	--	May 4	X	X	X	X

TABLE 1-II.- CALCULATED RADIATION EXPOSURES

Nuclide	Physical form	Critical organ		Total body, rem/μCi	Total, μCi	Total body, rem
		Organ	Exposure, rem/μCi			
Iodine-125	Albumin	Thyroid	0.0625 to 0.1875	0.00050	8	0.0040
Chromium-51	Chromate	Lung	.0020	.00036	75	.0270
Sulfur-35	Sulfate	Total body	.00009	.00009	100	.0090
Hydrogen-3	Water	Total body	.00017	.00017	175	.0298
Potassium-42	Chloride	Muscle	.00134	.00086	400	.3440
					Total	[a]0.4138

[a]Maximum permissible total body occupational exposure is 1.25 rems/quarter. This value can be increased to 3.0 rems/quarter if total for the year is less than 5.0 rems.

TABLE 1-III.- TWENTY-FOUR-HOUR URINE RESULTS

Sample time	Volume, ml	Epinephrine, µg/TV[a]	Norepinephrine, µg/TV	Aldosterone, µg/TV	Cortisol, µg/TV	17-OHCS[b], mg/TV	ADH, nU/TV	Osmolality, milliosmols	Electrolytes, meq/TV			
									Na	K	Cl	$^{c}H^{+}$
Commander												
F-30	2100	26.6	42.2	10.5	48.3	13.4	43.1	573	244	80	214	218
F-15	895	34.1	34.0	4.5	21.5	3.6	63.4	1003	138	65	117	409
F-5	1785	25.1	55.8	14.5	75.0	7.1	28.6	766	294	100	225	365
F-3	1090	17.6	35.1	2.4	33.7	5.0	37.2	712	159	72	142	114
F-2	1990	38.7	54.4	1.2	47.8	10.7	63.0	683	229	101	185	437
F-1	1250	18.7	40.3	17.1	37.5	3.8	42.5	790	166	86	141	217
In-flight	960	--	--	4.8	64.3	3.0	--	1192	159	94	150	--
R+1	815	12.0	40.1	3.6	45.6	1.3	31.8	826	57	42	46	217
R+2	1200	16.6	45.7	8.1	37.2	1.8	32.8	552	79	60	42	161
R+3	--	--	--	--	--	--	--	--	--	--	--	--
R+6	1775	17.2	37.4	2.8	17.7	4.4	12.4	505	114	43	73	248
Lunar module pilot												
F-30	2205	43.3	45.9	18.7	75.0	10.0	14.2	510	253	90	203	63
F-15	1640	55.7	59.3	5.1	70.5	12.0	10.2	629	175	52	143	446
F-5	2485	18.8	66.6	3.0	47.2	6.6	15.6	449	238	67	211	256
F-3	2835	22.1	55.8	10.6	35.4	6.5	28.1	355	154	65	130	223
F-2	2955	29.0	71.1	14.8	85.7	4.5	22.3	426	230	68	207	442
F-1	1540	22.9	63.2	13.0	57.8	2.4	23.0	743	189	82	179	313
In-flight	1265	--	--	8.7	124.0	2.9	--	1072	173	139	175	--
R+1	942	12.2	38.5	.8	67.4	3.6	301.3	697	85	41	56	205
R+2	1925	36.7	31.3	5.3	61.6	3.0	36.7	411	138	72	94	--
R+3	2890	91.3	63.9	7.0	115.6	11.1	75.8	376	196	82	165	173
R+6	3180	14.0	54.4	7.8	38.1	4.8	10.3	292	210	54	188	220
Command module pilot												
F-30	575	13.6	31.3	4.4	30.5	3.1	39.8	888	80	59	74	183
F-15	715	24.7	35.5	3.6	53.6	2.9	11.4	895	80	70	76	168
F-5	900	28.4	34.7	5.6	37.8	3.6	71.0	505	91	29	83	266
F-3	725	16.1	27.3	2.3	58.0	3.1	32.8	911	113	67	110	154
F-2	955	29.9	36.0	11.9	52.3	3.7	7.6	812	118	74	107	260
F-1	809	19.4	25.2	4.5	32.4	3.1	25.6	683	107	101	185	178
In-flight	495	--	--	3.7	30.7	3.1	--	1382	95	66	111	--
R+1	580	16.8	33.1	4.4	45.2	--	115.1	936	54	39	35	292
R+2	350	11.2	14.0	2.1	16.1	.8	4.7	761	23	13	12	169
R+3	1020	23.7	39.0	6.1	27.5	2.4	122.4	531	74	45	51	151
R+6	994	12.8	18.6	4.7	20.4	3.5	26.4	593	49	36	123	--

[a] Total volume.

[b] 17-hydroxycorticosteroid.

[c] Hydrogen ion.

TABLE 1-IV.- TOTAL AND FRACTIONATED KETOSTEROIDS

(a) CDR

Sample schedule	Total volume, ml	Fraction, mg/TV								Total, mg/TV
		PD	AND	Etio	DHEA	11=0 AND	11=0 Etio	11-OH AND	11-OH Etio	
F-30	2100	0.09	3.88	4.08	7.05	--	0.25	0.29	--	15.64
F-15	895	.33	4.37	4.24	4.11	--	.21	.33	--	13.59
F-5	1785	.21	2.83	2.65	2.65	--	.19	--	--	8.52
F-3	1090	.18	1.56	1.26	.92	--	.30	.08	--	4.30
F-2	1990	.36	3.77	3.59	1.77	--	.66	.36	--	10.50
F-1	1250	.22	2.38	2.01	2.01	--	.45	.25	--	7.33
Preflight mean ± SE[a]	1518 ± 206	.23 ± 0.04	3.13 ± 0.43	2.97 ± 0.49	3.09 ± 90	--	.34 ± 0.07	.26 ± 0.04	--	9.98 ± 1.7
In-flight	960	.65	4.03	3.47	8.58	--	.60	.58	0.56	18.47
R+1	815	.50	2.03	2.23	2.40	--	.40	.33	.38	8.26
R+2	1200	.39	1.96	1.83	1.06	0.03	.56	.27	.18	6.28
R+3	--	--	--	--	--	--	--	--	--	--
R+6	1775	.47	2.50	2.91	2.42	--	.29	--	.18	8.78

[a]Standard error.

TABLE 1-IV.- TOTAL AND FRACTIONATED KETOSTEROIDS - Continued

(b) CMP

Sample schedule	Total volume, ml	Fraction, mg/TV								Total, mg/TV
		PD	AND	Etio	DHEA	11=0 AND	11=0 Etio	11-OH AND	11-OH Etio	
F-30	575	0.12	0.72	2.05	0.08	--	0.47	0.31	0.17	3.92
F-15	715	.17	.79	2.27	.13	--	.47	.27	.26	4.36
F-5	900	.18	1.20	2.75	.11	--	.39	.16	.23	5.03
F-3	725	.25	1.23	2.02	.06	--	.44	--	--	4.01
F-2	955	.36	1.58	2.26	.21	--	.66	.21	.24	5.52
F-1	809	.29	1.15	1.56	.05	--	.58	.22	.31	4.15
Preflight mean ± SE	780 ± 56	.23 ± 0.04	1.11 ± 0.13	2.15 ± 0.16	.11 ± 0.02	--	.50 ± 0.04	.23 ± 0.03	.24 ± 0.02	4.50 ± 0.26
In-flight	495	.33	1.52	2.22	.08	--	.47	.14	.27	5.03
R+1	580	.23	1.17	1.60	.07	--	.72	.27	.30	4.35
R+2	--	--	--	--	--	--	--	--	--	--
R+3	1020	.17	1.05	1.60	.32	--	.27	--	--	3.41
R+6	994	.14	1.23	2.12	.10	--	.24	.10	--	3.92

TABLE 1-IV.- TOTAL AND FRACTIONATED KETOSTEROIDS - Concluded

(c) LMP

Sample schedule	Total volume, ml	Fraction, mg/TV								Total mg/TV
		PD	AND	Etio	DHEA	11=O AND	11=O Etio	11-OH AND	11-OH Etio	
F-30	2205	0.06	3.16	4.34	0.96	0.12	0.65	0.71	--	9.99
F-15	1640	.27	3.92	4.31	1.39	--	.53	--	0.50	10.93
F-5	2485	.08	1.94	2.34	1.25	--	.33	.21	--	6.14
F-3	2835	.15	1.58	2.44	.03	--	.23	--	--	4.44
F-2	2955	.22	2.87	2.78	.90	--	.58	.47	.43	8.24
F-1	1540	.18	3.04	3.85	1.49	--	.67	.43	.18	9.84
Preflight mean ± SE	2277 ± 243	.15 ± 0.03	2.75 ± 0.35	3.34 ± 0.38	1.00 ± 0.22	--	.50 ± 0.07	.46 ± 0.10	.37 ± 0.10	8.26 ± 1.03
In-flight	1265	.31	5.50	5.81	4.63	--	1.47	.76	1.05	19.53
R+1	942	.08	2.13	2.58	1.29	--	.36	--	.17	6.60
R+2	1925	.04	1.52	2.10	1.04	--	.22	--	--	4.92
R+3	2890	.13	2.81	3.51	1.77	--	.47	.20	--	8.90
R+6	3180	.34	2.82	4.23	1.33	--	.33	--	--	9.05

TABLE 1-V.- URINARY AMINO ACID PATTERNS

(a) CDR

Variable	Sample schedule							
	F-3	F-2	F-1	Preflight mean ± SE	In-flight	R+1	R+2	R+6
Volume, ml	1090	1990	1250	1375 ± 310	960	815	1200	5.36
Phosphoserine, mg/TV	10.92	24.07	13.52	16.17 ± 4.02	23.59	9.94	11.33	5.36
Phosphoethanolamine, mg/TV	3.14	7.40	4.19	4.91 ± 1.28	5.64	34.31	13.91	.65
Taurine, mg/TV	43.67	122.37	61.29	75.78 ± 23.85	178.10	127.96	157.29	42.30
Urea, g/TV	16.30	59.37	38.64	38.10 ± 12.44	44.28	35.02	18.35	13.44
Hydroxyproline, mg/TV	--	--	--	--	--	--	--	--
Aspartic acid, mg/TV	2.30	6.23	2.49	3.67 ± 1.28	4.77	2.12	2.26	1.04
Threonine, mg/TV	17.03	25.96	15.77	19.59 ± 3.21	34.08	14.46	20.23	5.68
Serine, mg/TV	31.53	54.65	32.83	39.67 ± 7.50	46.96	29.16	39.15	23.51
Asparagine/glutamine, mg/TV	65.79	127.55	73.06	88.80 ± 1.84	87.34	57.09	71.09	50.01
Sarcosine, mg/TV	--	--	--	--	9.79	--	--	--
Proline, mg/TV	--	--	--	--	6.02	--	--	--
Glutamic acid, mg/TV	2.73	6.41	2.11	3.75 ± 1.34	8.51	1.61	2.17	t[a]
Citrulline, mg/TV	--	--	--	--	0.52	--	--	--
Glycine, mg/TV	44.72	92.85	52.04	63.20 ± 15.00	243.88	37.68	66.03	41.37
Alanine, mg/TV	17.58	36.01	33.79	29.12 ± 5.80	33.00	17.37	34.87	14.95
α-aminoadipic acid, mg/TV	3.18	9.27	5.32	5.92 ± 1.78	8.56	5.94	6.63	t
α-amino-n-butyric acid, mg/TV	1.75	2.30	t	--	2.70	0.69	t	t
Valine, mg/TV	--	2.55	t	--	4.58	1.81	7.93	t
Half cystine, mg/TV	4.30	35.29	20.76	20.11 ± 8.95	9.54	17.67	10.88	t
Cystathionine, mg/TV	4.20	20.20	9.02	11.14 ± 4.74	10.49	9.35	19.54	10.65
Methionine, mg/TV	10.35	9.98	3.09	7.81 ± 2.36	5.71	4.02	7.66	5.96
Isoleucine, mg/TV	4.60	6.93	1.75	4.43 ± 1.50	8.08	3.81	7.16	4.62
Leucine, mg/TV	7.68	16.81	4.80	9.76 ± 3.62	10.38	6.42	7.79	7.68
Tyrosine, mg/TV	9.96	16.79	18.27	15.01 ± 2.56	3.02	14.00	16.77	11.47
Phenylalanine, mg/TV	7.90	11.19	7.40	8.83 ± 1.19	12.13	6.92	6.65	3.67
β-alanine, mg/TV	--	--	--	--	4.30	1.14	0.81	--
β-aminoisobutyric acid, mg/TV	2.60	--	6.62	--	10.39	3.33	3.34	5.96

[a]Trace.

TABLE 1-V.- URINARY AMINO ACID PATTERNS - Continued

(b) CMP

Variable	Sample schedule								
	F-3	F-2	F-1	Preflight mean ± SE	In-flight	R+1	R+2	R+3	R+6
Volume, ml	725	955	809	830 ± 67	495	580	350	1020	994
Phosphoserine, mg/TV	19.54	18.04	10.17	15.92 ± 2.91	12.32	14.25	5.43	9.14	7.60
Phosphoethanolamine, mg/TV	5.76	7.00	3.52	5.42 ± 1.02	3.41	4.78	1.34	1.33	1.09
Taurine, mg/TV	74.67	84.25	59.61	73.18 ± 7.44	57.59	151.18	64.68	112.81	14.05
Urea, g/TV	32.64	37.91	29.32	33.29 ± 2.50	17.62	27.44	11.82	13.60	8.46
Hydroxyproline, mg/TV	--	--	--	--	--	--	--	--	--
Aspartic acid, mg/TV	4.37	3.73	1.97	3.36 ± 0.72	2.47	2.72	0.97	1.71	1.29
Threonine, mg/TV	16.46	9.48	4.51	10.20 ± 3.51	7.92	11.21	4.34	8.87	8.72
Serine, mg/TV	33.26	30.33	20.52	28.04 ± 3.85	18.24	24.99	11.16	20.94	18.64
Asparagine/glutamine, mg/TV	58.76	52.57	39.83	50.39 ± 5.57	31.90	40.31	15.26	41.09	38.45
Sarcosine, mg/TV	--	--	--	--	9.95	--	--	--	--
Proline, mg/TV	--	--	--	--	--	--	--	--	--
Glutamic acid, mg/TV	3.73	4.82	5.39	4.65 ± 0.49	2.46	1.38	0.52	t	t
Citrulline, mg/TV	0.59	--	--	--	--	--	--	--	--
Glycine, mg/TV	81.61	67.93	50.74	66.76 ± 8.93	168.09	44.89	21.29	54.71	67.63
Alanine, mg/TV	22.40	23.46	13.73	16.53 ± 6.41	13.61	23.12	10.89	20.23	18.18
α-aminoadipic acid, mg/TV	4.73	5.46	8.74	6.31 ± 1.23	3.35	4.56	1.88	2.01	10.41
α-amino-n-butyric acid, mg/TV	0.08	t	0.69	--	0.39	0.62	--	--	--
Valine, mg/TV	--	4.07	2.27	--	1.84	1.16	1.50	t	--
Half cystine, mg/TV	6.27	5.79	5.83	5.96 ± 1.54	4.21	5.61	3.65	t	--
Cystathionine, mg/TV	5.85	9.73	12.08	9.22 ± 3.71	4.77	5.87	4.14	7.18	7.9
Methionine, mg/TV	7.90	4.72	4.42	5.68 ± 1.11	2.85	2.62	2.48	4.71	5.(
Isoleucine, mg/TV	4.90	3.53	3.04	3.83 ± 0.56	1.73	2.44	1.47	3.04	2.(
Leucine, mg/TV	6.16	5.77	4.59	5.51 ± 0.47	2.00	4.98	1.89	4.42	3.45
Tyrosine, mg/TV	22.23	14.40	9.64	15.42 ± 3.67	10.98	10.40	4.98	10.81	7.98
Phenylalanine, mg/TV	12.00	7.73	5.26	8.33 ± 1.97	6.19	6.17	2.86	5.19	2.59
β-alanine, mg/TV	--	--	--	--	--	0.13	--	--	--
β-aminoisobutyric acid, mg/TV	0.49	--	2.03	--	1.68	2.79	--	--	--

TABLE 1-V.- URINARY AMINO ACID PATTERNS - Concluded

(c) LMP

Variable	Sample schedule								
	F-3	F-2	F-1	Preflight mean ± SE	In-flight	R+1	R+2	R+3	R+6
Volume, ml	2835	2955	1540	2443 ± 453	1265	942	1925	2890	3180
Phosphoserine, mg/TV	10.72	12.00	16.45	13.06 ± 1.74	22.86	9.58	7.93	9.18	t
Phosphoethanolamine, mg/TV	5.67	4.68	5.61	5.32 ± 0.32	6.88	3.77	1.76	1.92	0.47
Taurine, mg/TV	46.25	208.68	137.68	164.20 ± 22.38	166.17	125.17	195.86	154.82	25.49
Urea, g/TV	27.59	30.50	48.62	35.57 ± 6.58	43.44	21.24	17.33	25.00	10.98
Hydroxyproline, mg/TV	--	--	--	--	--	--	--	--	--
Aspartic acid, mg/TV	2.93	2.67	4.91	3.50 ± 0.71	4.13	2.39	1.64	1.80	2.29
Threonine, mg/TV	9.30	12.05	13.68	11.68 ± 1.28	15.52	12.62	14.44	14.20	1.73
Serine, mg/TV	28.62	30.27	27.00	28.63 ± 0.94	35.50	22.40	26.51	31.79	11.97
Asparagine/glutamine, mg/TV	40.63	74.16	64.84	59.88 ± 9.99	64.45	44.67	55.22	68.57	22.85
Sarcosine, mg/TV	--	--	--	--	--	--	--	--	--
Proline, mg/TV	--	--	--	--	t	--	--	--	--
Glutamic acid, mg/TV	5.67	10.16	1.82	5.88 ± 2.41	5.78	1.47	t	t	--
Citrulline, mg/TV	--	--	--	--	--	--	--	--	--
Glycine, mg/TV	81.22	93.47	85.23	86.64 ± 3.61	263.89	51.94	82.01	82.36	39.64
Alanine, mg/TV	10.00	38.73	27.03	25.54 ± 8.33	30.28	20.41	28.99	35.39	9.82
α-aminoadipic acid, mg/TV	1.49	5.68	23.03	10.07 ± 6.59	8.52	5.08	3.34	t	t
α-amino-n-butyric acid, mg/TV	--	t	1.30	--	4.18	t	--	--	t
Valine, mg/	--	t	1.83	--	1.33	1.45	t	--	--
Half cystine, mg/TV	--	7.83	7.88	--	9.83	9.61	t	--	--
Cystathionine, mg/TV	16.39	17.62	16.76	16.92 ± 0.36	9.03	6.65	12.29	16.88	14.67
Methionine, mg/TV	10.47	10.65	5.58	8.90 ± 1.66	8.63	4.88	7.29	8.97	8.31
Isoleucine, mg/TV	8.40	5.80	8.12	7.44 ± 0.83	7.58	2.32	5.08	6.44	2.90
Leucine, mg/TV	6.64	8.68	17.10	10.81 ± 1.85	5.79	6.34	6.06	8.77	6.30
Tyrosine, mg/TV	9.67	23.30	32.82	21.93 ± 6.72	22.00	10.20	12.04	18.62	5.50
Phenylalanine, mg/TV	8.75	1.19	19.75	9.90 ± 5.39	7.13	3.80	3.51	4.59	4.66
β-alanine, mg/TV	--	--	--	--	2.21	0.87	--	t	--
β-aminoisobutyric acid, mg/TV	3.53	--	7.02	--	3.11	0.59	0.47	t	--

TABLE 1-VI.- SERUM/PLASMA RESULTS

Sample time	Hydrocortisone, µg/100 ml	Angiotensin I, ng/ml/hr	T_3, percent	T_4, µg/100 ml	Electrolytes, meg/liter			Osmolality, milliosmols	Insulin, µU/ml	Parathyroid hormone, ng/ml	ACTH, pg/ml	HGH, ng/ml
					Na	K	Cl					
CDR												
F-30	14.0	0.43	33.1	7.6	142	4.9	107	295	16	--	49.5	0.7
F-15	18.4	1.15	33.8	4.3	141	4.6	107	290	15	0.66	32.4	1.6
F-5	10.0	3.13	34.6	5.1	140	4.7	105	286	--	--	69.5	1.6
ASAP	8.8	11.60	31.2	5.6	145	4.0	110	300	12	.64	12.0	1.6
R+1	10.0	1.46	35.8	6.4	142	4.2	106	292	14	.63	26.2	.5
R+3	13.6	.71	35.4	--	139	4.7	107	286	--	.71	139.0	1.6
R+6	11.0	1.33	32.3	6.2	141	4.7	111	299	--	.66	23.0	1.2
LMP												
F-30	27.6	1.71	32.7	5.9	142	4.6	101	292	20	--	33.5	0.7
F-15	16.8	.90	--	5.3	144	4.5	106	288	11	<0.42	25.8	4.0
F-5	11.8	1.30	--	7.3	140	3.9	98	285	--	--	68.8	4.4
ASAP	9.0	2.46	32.3	5.6	146	4.2	105	303	12	<.42	38.1	4.8
R+1	8.8	.54	35.8	5.7	146	4.2	106	301	10	<.42	15.9	4.4
R+3	13.6	4.33	35.0	4.5	141	3.9	103	291	17	<.42	16.1	2.8
R+6	16.0	.50	31.5	5.9	141	3.9	103	294	14	<.42	12.0	1.2
CMP												
F-30	18.8	0.41	33.1	4.8	144	4.5	106	295	16	--	17.7	2.4
F-15	25.0	2.44	--	5.8	140	4.1	102	288	17	0.46	17.4	1.6
F-5	12.6	1.56	--	4.7	141	4.2	103	288	--	--	83.6	6.8
ASAP	10.4	6.86	31.9	--	145	3.5	106	300	23	.52	<1.0	44.0
R+1	9.2	1.06	35.4	5.9	142	3.9	105	295	16	.68	9.4	2.0
R+3	26.8	1.96	36.5	5.0	143	4.4	108	290	9	<.42	27.4	1.6
R+6	15.6	.29	28.1	4.0	143	3.8	107	295	22	<.42	13.2	1.8

TABLE 1-VII.- BODY FLUID VOLUMES

Schedule	Flight crewman			Controls		
	CDR	LMP	CMP	Subject A	Subject B	Subject C
Red-cell mass, ml						
F-15	2154	1976	2043	2005	1689	2060
ASAP	1861	1740	1695	1970	1676	2119
R+7	1822	1802	1764	1986	1722	2028
Plasma volume, ml						
F-15	3085	3094	3060	3460	2500	3320
ASAP	2813	3138	2800	3840	3070	3992
R+1	3175	3336	3012	3894	3078	4049
R+7	3198	3323	3334	3941	3254	4054
Total body water, liters						
F-15	49.2	48.2	43.6	48.7	46.0	49.3
ASAP	47.8	46.5	41.7	48.0	45.4	47.6
R+1	48.9	47.5	43.1	47.5	45.3	47.4
R+7	--	48.0	44.0	48.6	46.3	50.1
Extracellular fluid, liters						
F-15	15.5	14.4	13.9	17.6	17.0	16.3
ASAP	15.1	14.7	13.2	18.2	17.9	17.8
R+1	15.9	15.1	--	17.9	18.1	17.6
R+7	15.2	14.3	13.9	17.8	17.4	16.5

TABLE 1-VIII.- FLUID VOLUMES/kg BODY WEIGHT

Schedule	Flight crewmen			Controls		
	CDR	LMP	CMP	Subject A	Subject B	Subject C
Plasma volume, ml/kg						
F-15	38.3	41.9	49.4	46.7	32.5	40.5
ASAP	37.2	44.6	47.8	54.2	41.8	49.7
R+1	41.4	46.5	50.2	55.0	41.9	50.4
R+7	42.3	46.2	54.4	53.6	42.4	49.9
Total body water, ml/kg						
F-15	610	652	703	657	597	601
ASAP	632	659	712	678	618	592
R+1	638	662	720	671	616	589
R+7	--	666	718	661	604	617
Extracellular fluid, ml/kg						
F-15	192	195	224	238	221	199
ASAP	200	208	225	257	244	221
R+1	207	210	--	253	246	219
R+7	201	199	227	242	227	203

TABLE 1-IX.- DERIVED VOLUMES

Schedule	Flight crewmen			Controls		
	CDR	LMP	CMP	Subject A	Subject B	Subject C
Intracellular fluid, liters						
F-15	33.7	33.8	29.7	31.1	29.0	33.0
ASAP	32.7	31.8	28.5	29.8	27.5	29.8
R+1	33.0	32.4	--	29.6	27.2	29.8
R+7	--	33.7	30.1	30.8	28.9	33.6
Interstitial fluid, liters						
F-15	12.4	11.3	10.8	14.1	14.5	13.0
ASAP	12.3	11.6	10.4	14.4	14.8	13.8
R+1	12.7	11.8	--	14.1	15.0	13.6
R+7	12.0	11.0	10.6	13.9	14.2	12.5
Intracellular fluid, ml/kg						
F-15	418	457	479	420	377	402
ASAP	432	450	486	421	374	371
R+1	430	452	--	418	370	371
R+7	--	468	491	419	377	414
Interstitial fluid, ml/kg						
F-15	154	153	174	190	188	158
ASAP	163	164	177	203	201	172
R+1	166	164	--	199	204	169
R+7	159	153	173	189	185	154

TABLE 1-X.- TOTAL BODY K

Schedule	Flight crewmen			Controls		
	CDR	IMP	CMP	Subject A	Subject B	Subject C
	meq K/kg					
F-30	47.3	47.6	49.8	46.7	43.3	44.8
F-15	46.0	47.0	49.3	46.6	42.4	44.0
F-5	48.2	48.2	51.4	45.2	42.4	43.8
ASAP	49.2	53.1	53.8	45.4	42.0	45.1
R+7	48.1	46.8	49.2	46.6	43.0	45.7
	Total meq K					
F-30	3828	3485	3148	3446	3401	3712
F-15	3707	3473	3055	3454	3263	3606
F-5	3808	3501	3220	3309	3268	3609
ASAP	3773	3807	3223	3214	3087	3626
R+7	3636	3370	3016	3425	3298	3711

2. CHANGES IN GLUCOSE, INSULIN, AND GROWTH HORMONE LEVELS ASSOCIATED WITH BED REST

By Joan Vernikos-Danellis, Ph. D., Carolyn S. Leach, Ph. D., Charles M. Winget, Ph. D., Anne L. Goodwin, and P. C. Rambaut, Ph. D.

Investigators have recently reported that the cardiovascular deconditioning and the alterations in plasma volume that result following exposure to bed rest without exercise for 2 weeks are accompanied by impaired tolerance to a glucose load and by excessive plasma insulin responses to glucose tolerance tests (ref. 2-1).[1] Similarly, various degrees of physical inactivity have, in the past, been reported by several investigators to result in an apparent inefficient handling of glucose. Lutwak and Whedon (ref. 2-2) found that complete bed rest for 1 to 3 weeks resulted in decreased glucose utilization. Blotner (ref. 2-3), studying 70 nondiabetic adults and 16 children confined to bed for 1 month to 8 years, found excessive increases in blood glucose following a 100-gram oral glucose load, the effects being most pronounced in those confined the longest. In addition, Naughton and Wulff (ref. 2-4) found that the insulin response to glucose load in sedentary men is much greater than in active persons but that the disappearance of glucose is the same. In none of these studies, however, were resting levels of plasma glucose or insulin reported to be altered. This study was designed to determine the changes in the circulating levels of insulin, glucose, and human growth hormone (HGH) in young, healthy male subjects exposed to bed rest for 56 days.

The same five male subjects and the same daily schedule and experimental conditions were used as described by Winget et al. (sec. 3). Blood samples were assayed for HGH, insulin, and glucose before bed rest; at 10, 20, 30, 42, and 54 days after confinement to bed; and at 10 and 20 days after the subjects had again been ambulatory. Human growth hormone and insulin were determined by radioimmunoassay techniques, and plasma glucose was determined by the autoanalyzer ferric-cyanide method. The data were analyzed by the usual statistical techniques (ref. 2-5).

Figure 2-1 shows the mean circulating HGH levels per 48-hour sampling period before, during, and after 56 days of bed rest. Each point represents the mean of twelve 4-hourly samples per 48-hour period for the group of five subjects. Plasma HGH showed an initial drop at 10 days of bed rest, then rose significantly ($P < 0.05$) at 20 days (1.5-fold increase) and subsequently decreased gradually, reaching levels (2.5 mg/ml) well below pre-bed-rest control

[1]Also: C. B. Dolkas, unpublished observations, 1972.

levels (4.2 mg/ml) at 54 days of bed rest. The changes in mean daily plasma, glucose, and insulin levels are shown in figure 2-2. Glucose concentrations remained unchanged in spite of a marked increase in mean daily insulin levels during the first 30 days of bed rest. With continued exposure to bed rest, insulin began decreasing toward pre-bed-rest levels and glucose also decreased below control levels. By day 54 of bed rest, glucose reduction was significant ($P < 0.05$), reaching a level of 75 mg/100 ml/24 hr and recovering during the control period.

The daily mean changes are not generally due to an overall increase in hormone or glucose levels at all times of the day, but reflect a change in the amplitude of the diurnal variation. Figures 2-3, 2-4, and 2-5 show the diurnal rhythms in plasma HGH, glucose, and insulin, respectively, at various intervals during the study. Each point represents the mean concentration in the subjects. Growth hormone showed a significant daily fluctuation (fig. 2-3) with peak levels occurring at 2000 and 0400 hours. An initial reduction in amplitude at 10 days of bed rest was followed by considerable increases in amplitude at 20 and 30 days; subsequently, the rhythm almost disappeared on days 42 and 54. Although there was considerable variability of the time at which the peak occurred, it was always associated with the late evening and night samples; the concentration of HGH was always lowest at 0730 hours, just before the lights were turned on. This association was also true for the plasma glucose and insulin rhythms (figs. 2-4 and 2-5). The amplitude of the glucose rhythm also varied throughout the study; and on day 54, when the daily mean showed a significant decrease, the blood glucose concentration at 1600 hours was as low as 62.2 mg/100 ml. The mean increase in circulating insulin levels was due to the increased amplitude of its diurnal rhythm (fig. 2-5). The peak insulin level shifted to a time earlier in the day and was maintained at a higher level during the "lights on" phase for the first 30 days of bed rest. After the 30th day, the amplitude began to decrease toward pre-bed-rest levels.

The effect of bed rest on the amplitude of diurnal rhythms has been observed for other hormones also. Because exercise has been found to stimulate HGH secretion (ref. 2-6) without altering blood glucose levels, the initial drop in blood HGH may be related to the relative inactivity of bed rest. The subsequent rise in HGH may be secondary to the increased insulin levels and may contribute to the impaired glucose tolerance reported by others (ref. 2-1) and to the relative ineffectiveness of the rising levels of insulin on blood glucose observed in this study. However, the increased levels of cortisol during the first 30 days of bed rest may also be contributing to the decreased glucose tolerance and the raised plasma insulin levels by decreasing peripheral utilization of glucose through the inhibition of glucose phosphorylation in muscle and adipose tissue. It is also possible that the apparent insulin insensitivity may be attributed to increases in the radioimmunoassayable hormone level and may not reflect biological activity. Levels of radioimmunoassayable and biological activity of various hormones have recently been found to differ, and it has been proposed that alterations in the molecular configuration of the hormone may account for these discrepancies.[2]

---

[2]C. S. Nicoll, personal communication, 1972.

In general, these prolonged studies have revealed that the endocrine picture during the first 3 weeks of bed rest (which may be partially attributed to early changes in plasma volume) appears to be quite different from that which develops after more prolonged exposure to bed rest (table 2-I). It appears imperative to extend the experimental period beyond 56 days and to establish the mechanisms responsible for the alterations in glucose homeostasis.

REFERENCES

2-1. Piemme, Thomas E.: Effects of Two Weeks of Bed Rest on Carbohydrate Metabolism. Sec. 21 of Hypogravic and Hypodynamic Environments, NASA SP-269, 1971.

2-2. Lutwak, L.; and Whedon, G. D.; The Effect of Physical Conditioning on Glucose Tolerance. Clin. Res., vol. 7, 1959, p. 143.

2-3. Blotner, Harry: Effect of Prolonged Physical Inactivity on Tolerance of Sugar. Arch. Intern. Med., vol. 75, no. 1, Jan. 1945, pp. 39-44.

2-4. Naughton, John; and Wulff, Johan: Effect of Physical Activity on Carbohydrate Metabolism. J. Lab. Clin. Med., vol. 70, no. 6, Dec. 1967, p. 996.

2-5. Bliss, Chester Ittner: The Statistics of Bioassay, with Special Reference to Vitamins. Academic Press (New York), 1952.

2-6. Hunter, W. M.; Fonseka, C. C.; and Passmore, R.: Growth Hormone: Important Role in Muscular Exercise in Adults. Science, vol. 150, no. 3699, Nov. 19, 1965, pp. 1051-1053.

TABLE 2-I.- SUMMARY OF CHANGES IN ENDOCRINE PARAMETERS DURING 56 DAYS OF BED REST

Secretion	First 3 weeks	Last 3 weeks
Insulin	2.5× increase	Decrease toward control levels
Glucose	No change	Decrease below control levels
HGH	1.5× increase	Decrease below control levels
Cortisol	2× increase	Decrease below control levels
ACTH[a]	No change	3× increase over control levels

[a]Adrenocorticotrophic hormone.

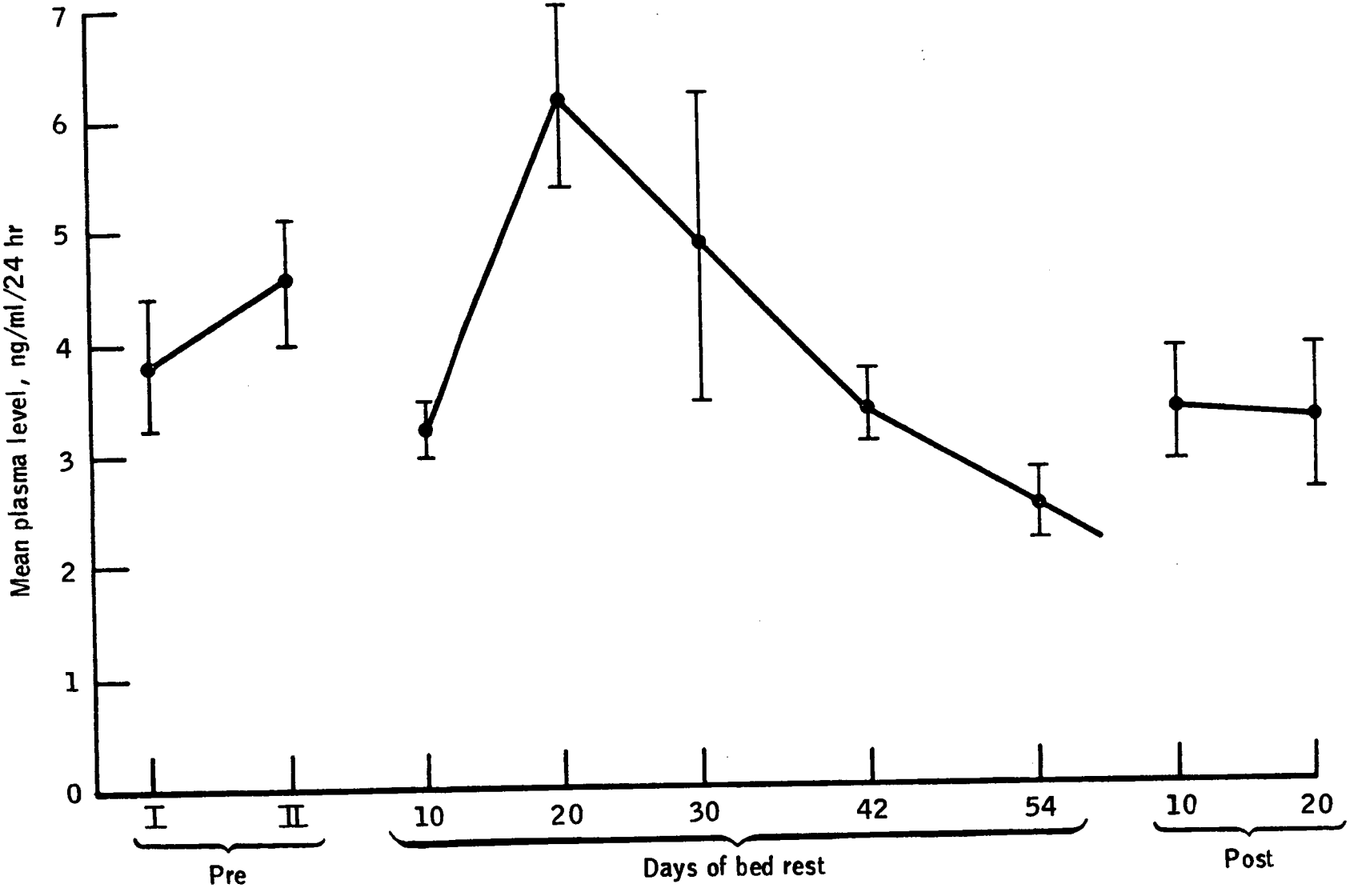


Figure 2-1.- Mean circulating HGH levels per 48-hour sampling period before, during, and after 56 days of bed rest. Brackets represent mean ± standard error (N = 5).

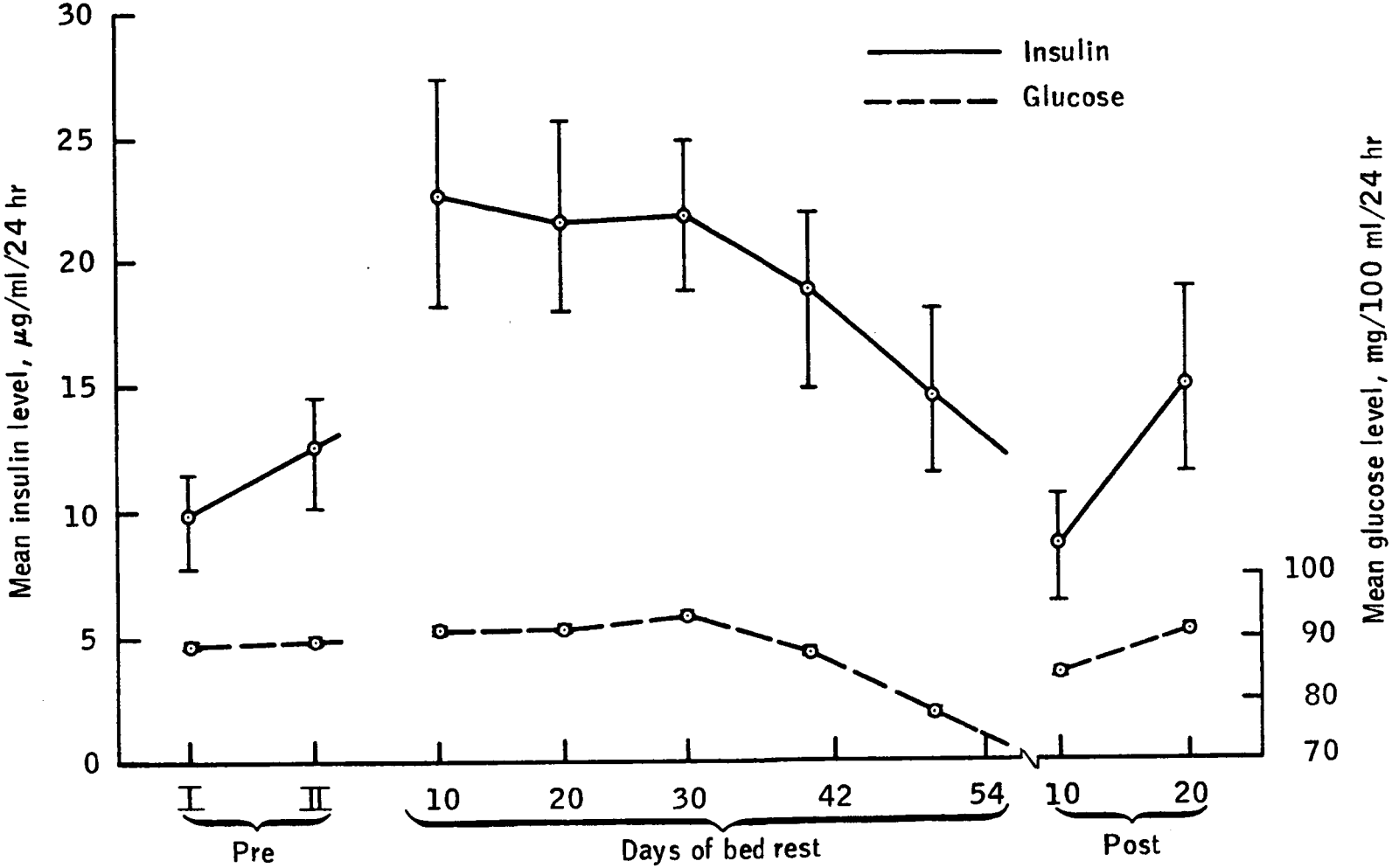


Figure 2-2.- Mean circulating insulin and glucose levels per 48-hour sampling period before, during, and after 56 days of bed rest. Brackets represent mean ± standard error (N = 5).

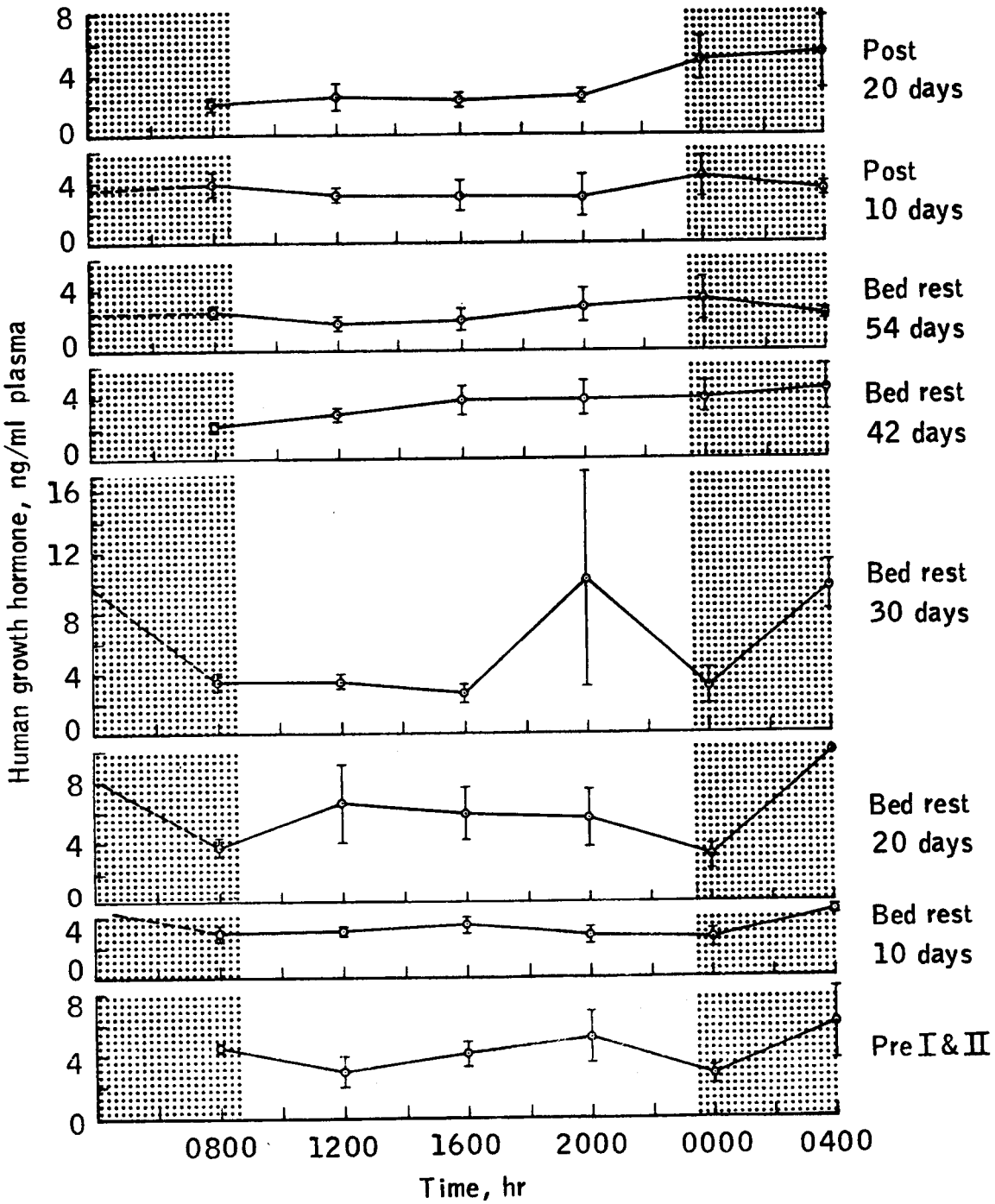


Figure 2-3.- Diurnal rhythms in mean circulating HGH levels before, during, and after 56 days of bed rest. Brackets represent mean ± standard error (N = 5); stippled area represents lights-off period.

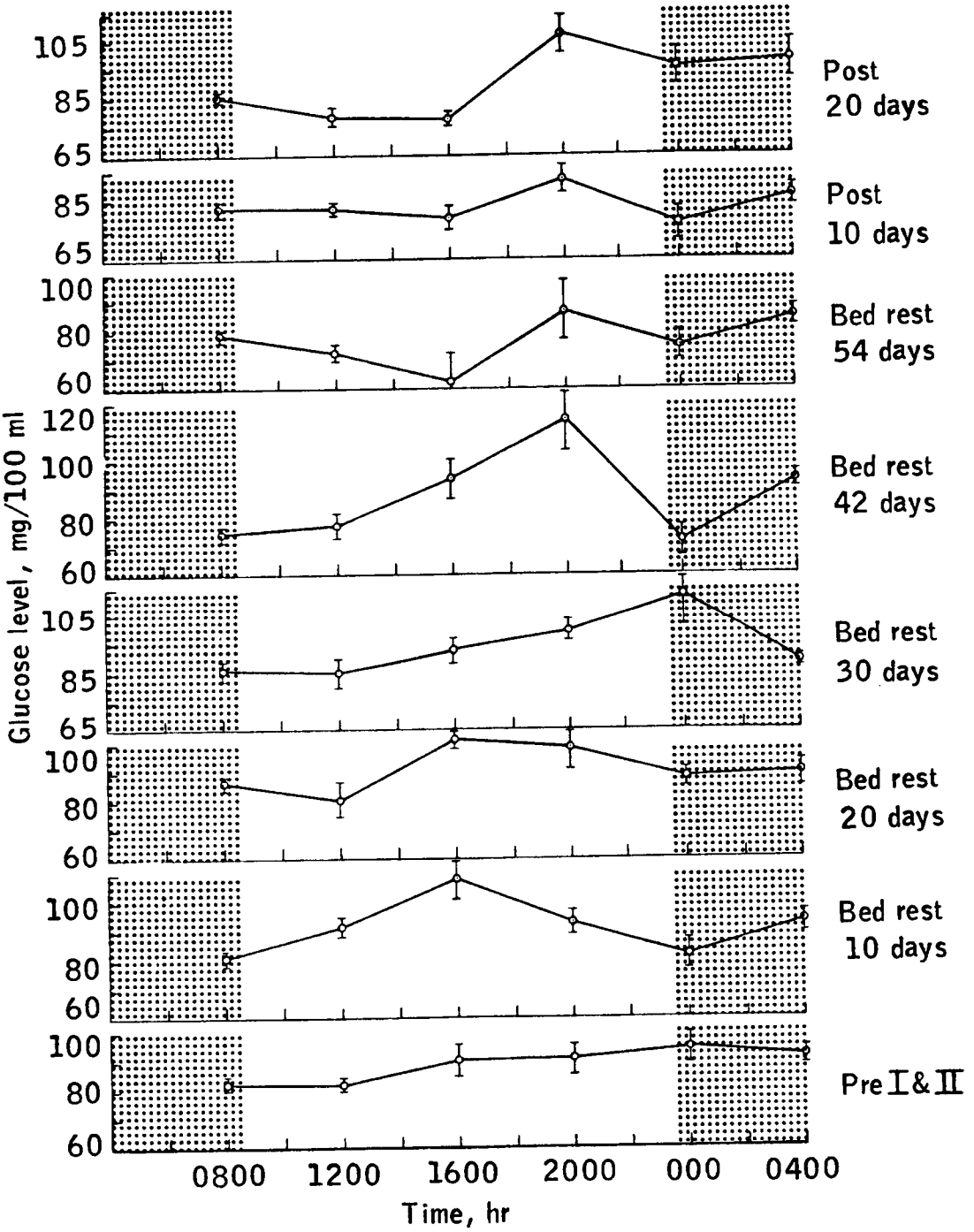


Figure 2-4.- Diurnal rhythms in mean circulating glucose levels before, during, and after 56 days of bed rest. Brackets represent mean ± standard error (N = 5); stippled area represents lights-off period.

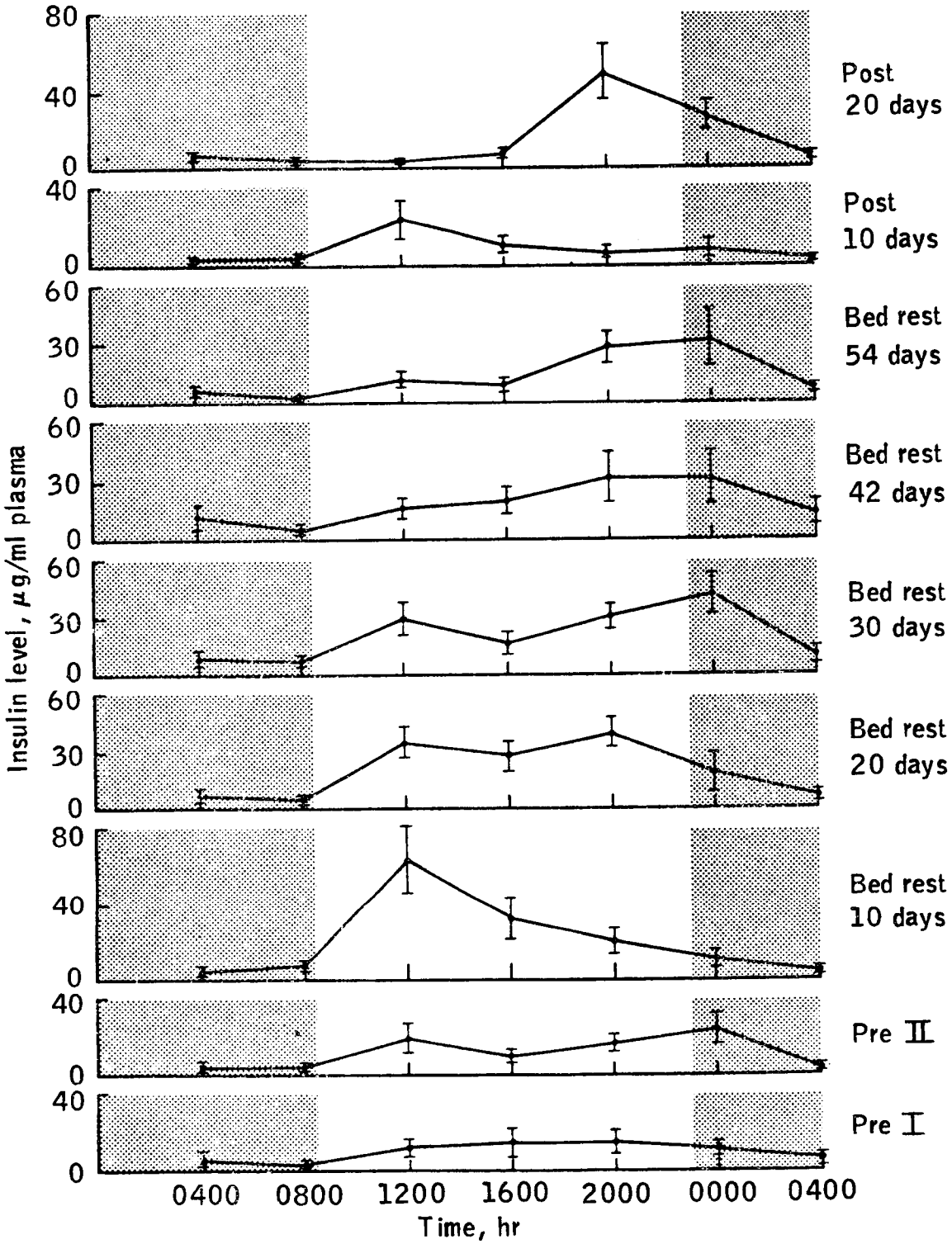


Figure 2-5.- Diurnal rhythms in mean circulating insulin levels before, during, and after 56 days of bed rest. Brackets represent mean ± standard error (N = 5); stippled area represents lights-off period.

3. FIFTY-SIX DAYS OF BED REST:

CIRCADIAN RHYTHMS OF HEART RATE AND BODY TEMPERATURE

By Charles M. Winget, Ph. D., Joan Vernikos-Danellis, Ph. D.,
C. W. DeRoshia, S. E. Cronin, Carolyn S. Leach, Ph. D.,
and Paul C. Rambaut, Ph. D.

The two basic factors that act on the normal subject during bed rest are restriction of muscular activity (hypokinesis) and a characteristic redistribution of blood because of a change in hydrostatic pressure (ref. 3-1). Bed rest for varying periods with healthy individuals has been found to lead to venous thrombosis, hypostatic pneumonia, a reduction in heart volume, and increases in resting and work pulses (ref. 3-2). Other studies have revealed reductions in circulating blood volume, muscle atrophy, and negative nitrogen and mineral balances (refs. 3-3 to 3-5). Most of this work reported that hypokinesis had a significant role in the etiology of the reported disorders. The characteristic time structure of these biological processes and their importance in regulatory mechanisms were not considered.

If the central nervous system plays any role in the development of the bed-rest symptoms, then it might be expected that circadian synchrony, which is also regulated by central mechanisms (endogenous synchronizers), would be disturbed, and that it would be disturbed in spite of maintaining subjects in an environment with well-defined exogenous synchronizers (light, meals, etc.).

The two bed-rest studies reported here evaluated the influence on circadian waveform as a result of removing such factors as posture and activity; subjects were healthy human males in a well-regulated environment with a fixed photoperiod.

RESULTS

Study I indicated that all subjects showed a stable phase and amplitude during the 6 days before bed rest, with the maximum occurring in the latter half of the light period. Despite this homogeneity of data and despite the continuation of the regular photoperiod of 14 hours of light and 10 hours of dark (14:10 LD) and the feeding at regular hours, there was a tendency, when the subjects were put to bed, for the ear canal temperature (body temperature (BT)) rhythm to become desynchronized with the environment although it remained circadian. An example of this desynchronization is the sequential information of subject 6A (fig. 3-1).

Bed rest also produced a depression in the mean BT that did not return to the pre-bed-rest values. A change in the amplitude and time of peak of the

heart rate (HR) rhythm was also noted during the period after bed rest. The daily range of BT was about 1 K (1° C) and the HR was about 15 beats/min. The nonexercised group had less stable circadian oscillations than the exercised group. Study II confirmed and expanded these findings.

Because the summation dial method for analyzing nonstationary data assumes a period of 24 hours, it was important to determine that this was indeed so under the present experimental conditions and that bed rest was not affecting the circadian period of these parameters. Comparison of the individual periodograms of the ambulatory and bed-rest subjects indicates the presence of a circadian period in both cases.

The HR data for the bed-rest group can be divided into four segments.

1. A pre-bed-rest period of stable rhythms similar to those of the ambulatory group

2. A small phase shift (approximately 2 hours) after the first 3 weeks of bed rest

3. A second phase shift (approximately 4 hours) that occurred after approximately 20 days of bed rest (day 40 of the experiment) and that continued for the remaining 5 weeks of bed rest

4. A post-bed-rest phase in which subjects almost immediately resynchronized with their original pre-bed-rest rhythms.

The summation dials for the BT of the ambulatory and bed-rest subjects are shown in figures 3-2 and 3-3, respectively. The BT of the ambulatory group was somewhat less stable than their HR rhythms. However, the peaks for all subjects occurred in the same time quadrant (i.e., between 2045 and 0245 hours) as indicated by the direction of the train of vectors. During bed rest, the BT data varied even more. Three bed-rest subjects peaked in the same quadrant as the ambulatory subjects, and the other three peaked at approximately 0600 hours (60° out of phase). All subjects showed considerable phase changes throughout the bed rest; three of them showed random shifts that represent rhythm asynchrony. Only two subjects resynchronized relative to base at the end of the 3-week post-bed-rest period.

Because the sharpest phase shift in the HR rhythm appeared to occur on approximately day 20 of bed rest, the data were scanned to determine more precisely the actual time and day at which the shift occurred (fig. 3-4). Four bed-rest subjects showed the sharpest phase shift on day 23 of bed rest (day 43 of the experiment) whereas the other two bed-rest subjects showed a similar change on day 24 of bed rest (day 44 of the experiment). On those days, the HR of these bed-rest subjects increased sharply to 87 beats/min by 0100 hours and did not reach its lowest point (56 beats/min) until 0700 hours. In contrast, no ambulatory subjects showed an increase in HR at these hours; in fact, their HR decreased from 74 beats/min at 2300 hours to 62 beats/min at 0100 hours.

The BT rhythm did not show a sudden, single phase shift as did the HR. Figure 3-5 compares the daily phase angle, the daily integrated amplitude and the daily mean of the BT data of both groups. The results indicate that numerous phase shifts occurred in the bed-rest subjects compared to the relatively unchanging phase angle of the ambulatory controls and of the same bed-rest subjects during their ambulatory periods. The mean daily BT decreased progressively in spite of an unchanged integrated amplitude, indicating that the BT rhythm fluctuated in bed rest with the same amplitude about a new lower mean level. The decrease in mean daily BT had not recovered by the end of the 21-day post-bed-rest period.

DISCUSSION

It has generally been agreed that light acts as the primary influence in maintaining synchrony of circadian rhythms (refs. 3-6 to 3-12). Research on the properties and characteristics of rhythms has concentrated on producing rhythm desynchronization by manipulating the photoperiod (refs. 3-13 and 3-14). Even the search for secondary synchronizers such as temperature and magnetic fields has involved analysis of the influence of these variables in the absence of light cues; that is, either continuous light or continuous dark environments (refs. 3-15 to 3-17). Study I investigated the physiological changes that occur in man in response to prolonged bed rest; it was observed that desynchronization of some circadian rhythms occurred in spite of the fact that the subjects were maintained in a highly structured environment including a controlled photoperiod of 14:10 LD. Because such desynchronization in the presence of a defined light environment has not been described previously in healthy subjects, it was important to first confirm this finding and, secondly, to determine if the change induced by bed rest was sufficiently powerful to cause rhythm asynchrony in spite of the unchanged photoperiod.

The results of study II confirmed the previous findings that the primary influence of bed rest on BT and HR rhythms is to reduce the amplitude and change their phase relationships. The normally entrained rhythms were altered after approximately 20 days of bed rest, when they lost their normal relationship to the photoperiod and to each other. In addition, bed rest induced a depression of BT and an initial bradycardia. The possibility that this rhythm asynchrony may have been due to the inactivity associated with bed rest was ruled out in bed-rest study I, where a moderately heavy exercise regimen did not prevent these changes. Study II also rules out the possibility that the prolonged confinement associated with this type of experiment may have been causing the observed phase shift, because the ambulatory control subjects showed none of the changes observed in the bed-rest subjects.

The dissociation of the HR and BT rhythms from each other and from the light schedule during bed rest and the prompt reassociation of the two rhythms in the post-bed-rest ambulatory period suggest that synchrony of these rhythms may be dependent on posture. Therefore, it seems reasonable that the postural

change involved in bed rest or some physiological consequence of that, such as hydrostatic pressure changes or redistribution of body fluids and electrolytes, may be primarily responsible for the rhythm asynchrony.

Various rhythms in man have been reported to be dependent on different cues. For example, it has been suggested that the rhythm in aldosterone excretion is primarily posture-dependent, while the rhythmicity of other parameters such as plasma cortisol levels appears to be unaffected by bed rest and remains entrained to the light-dark cycle. On the other hand, HR and, to a lesser extent, BT appear to require both light and other (postural) cues to maintain their rhythm synchrony.

The level of the baseline, about which the homeostatic mechanisms operate, varies rhythmically; and this rhythmicity is controlled by exogenous and endogenous synchronizers. Since the time of Claude Bernard, physiologists have emphasized the study of the mechanisms by which organisms maintain the relative constancy of their internal environment in response to change in the external environment. Such change or stress has generally been considered as an increase in magnitude or duration of inputs to the system. With the exception of behavioral and biologic rhythm research, little attention has been given to the biological consequences of an absence of or reduction in input stimuli. The psychological consequences of isolation, the disturbance of circadian rhythms in an environment where light intensity was below a certain threshold, and the clinical consequences of prolonged bed rest in hospitalized patients have long been recognized. Studies on the effect of prolonged bed rest suggest that neither reduced activity alone nor relative confinement alone (both examples of low-input environments) result in HR and BT rhythm asynchrony. On the other hand, it appears that the reduction in input stimuli to proprioceptive receptors resulting from the postural change alone, or in addition to the confinement and inactivity inherent to prolonged bed rest, was responsible for the observed rhythm asynchrony. The results further support the hypothesis that maintaining circadian rhythm synchrony is not dependent on light alone as the environmental synchronizer. It is more likely that a variety of input stimuli is required to attain a certain threshold before synchrony of these rhythms with the environment and with each other can be maintained. If this hypothesis is true, it should be possible to maintain rhythm synchrony in bed rest by changing light intensity or other social and environmental stimuli.

REFERENCES

3-1. Vogt, Fred B.; Mack, Pauline Berry; and Johnson, Philip C.: Tilt Table Response and Blood Volume Changes Associated with Thirty Days of Recumbency. Aerospace Med., vol. 37, no. 8, Aug. 1966, pp. 771-777.

3-2. Rodahl, K.; Birkhead, N. C.; Blizzard, J. J.; Issejutz, B., Jr.; and Pruett, E. D. R.: Physiological Changes During Prolonged Bedrest. Nutrition and Physical Activity, G. Blix, ed., Aluquist and Wilsells Uppsala, 1967, pp. 107-114.

3-3. Lamb, Lawrence E.; and Stevens, Paul M.: Influence of Lower Body Negative Pressure on the Level of Hydration During Bed Rest. Aerospace Med., vol. 36, no. 12, Dec. 12, 1965, pp. 1145-1151.

3-4. Taylor, Henry Longstreet; Erickson, Lester; Henschel, Austin; and Keys, Ancel: The Effect of Bed Rest on the Blood Volume of Normal Young Men. Am. J. Physiol., vol. 144, no. 2, July 1, 1945, pp. 227-232.

3-5. Taylor, Henry Longstreet; Henschel, Austin; Brožek, Josef; and Keys, Ancel: Effects of Bed Rest on Cardiovascular Function and Work Performance. J. Appl. Physiol., vol. 2, no. 5, Nov. 1949, pp. 223-239.

3-6. Axelrod, J.; Wurtman, R. J.; and Winget, C. M.: Melatonin Synthesis in the Hen Pineal Gland and Its Control by Light. Nature, vol. 201, no. 4924, Mar. 1964, p. 1134.

3-7. Aschoff, J.: Comparative Physiology: Diurnal Rhythms. Ann. Rev. Physiol., vol. 25, 1963, pp. 581-600.

3-8. Winget, C. M.; and Card, D. H.: Daily Rhythm Changes Associated with Variations in Light Intensity and Color. Life Sciences and Space Research V., North-Holland Publishing Co. (Amsterdam), 1967, pp. 148-158.

3-9. Winget, C. M.; Rahlmann, D. F.; and Pace, N.: Phase Relationships Between Circadian Rhythms and Photoperiodism in the Monkey. Circadian Rhythms in Non-Human Primates, Frederick H. Rohles, ed., Basel, Switz. Karger (New York), 1969, pp. 64-74.

3-10. Winget, C. M.; Rosenblatt, L. S.; DeRoshia, C. W.; and Hetherington, N. W.: Mechanisms of Action of Light on Circadian Rhythms in the Monkey. Life Science and Space Research, vol. 8, 1970, pp. 247-258.

3-11. Bünning, Erwin: The Physiological Clock. Revised second ed., Springer-Verlag (New York), 1967.

3-12. Flink, Edmund B.; and Doe, Richard P.: Effect of Sudden Time Displacement by Air Travel on Synchronization of Adrenal Function. Proc. Soc. Exp. Biol. Med., vol. 100, 1959, pp. 498-501.

3-13. Halberg, Franz; Albrecht, Paul G.; and Barnum, Cyrus P., Jr.: Phase Shifting of Liver-Glycogen Rhythm in Intact Mice. Am. J. Physiol., vol. 199, no. 3, Sept. 1960, pp. 400-402.

3-14. Wever, R.: Einfluss Schwacher Elektromagnetischer Felder auf die Circadiane Periodik des Menschen. Naturwiss., vol. 55, no. 1, 1968, pp. 29-32.

3-15. Wilson, F.; and Snowball, G. J.: Some Effects of Temperature on Diurnal Periodicity of Adult Emergence in Trichopoda Pennipes (Diptera Tachimdae). Australian J. Zool., vol. 7, 1959, pp. 1-6.

3-16. Aschoff, J.; Fatranska, M.; Giedke, H.; Doerr, P.; Stamm, D.; and Wisser, H.: Human Circadian Rhythms in Continuous Darkness: Entrainment by Social Cues. Science, vol. 171, no. 3967, Jan. 15, 1971, pp. 213-215.

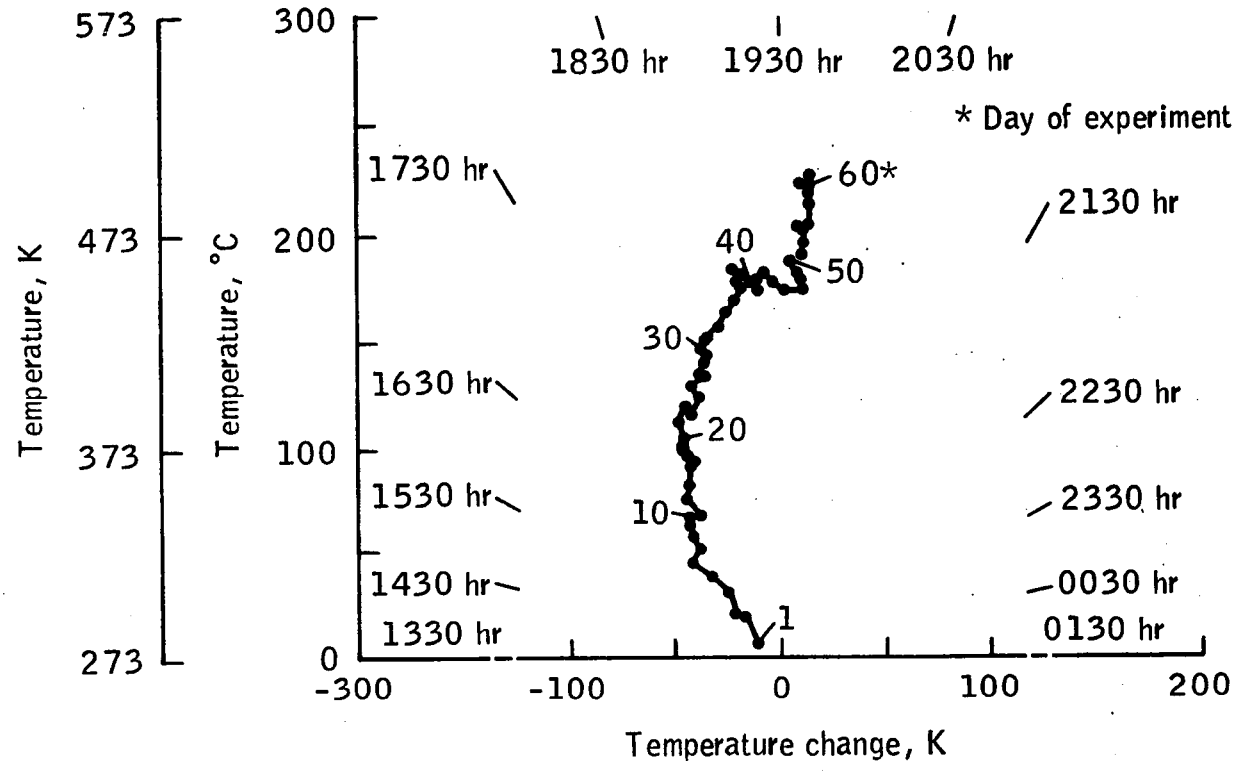
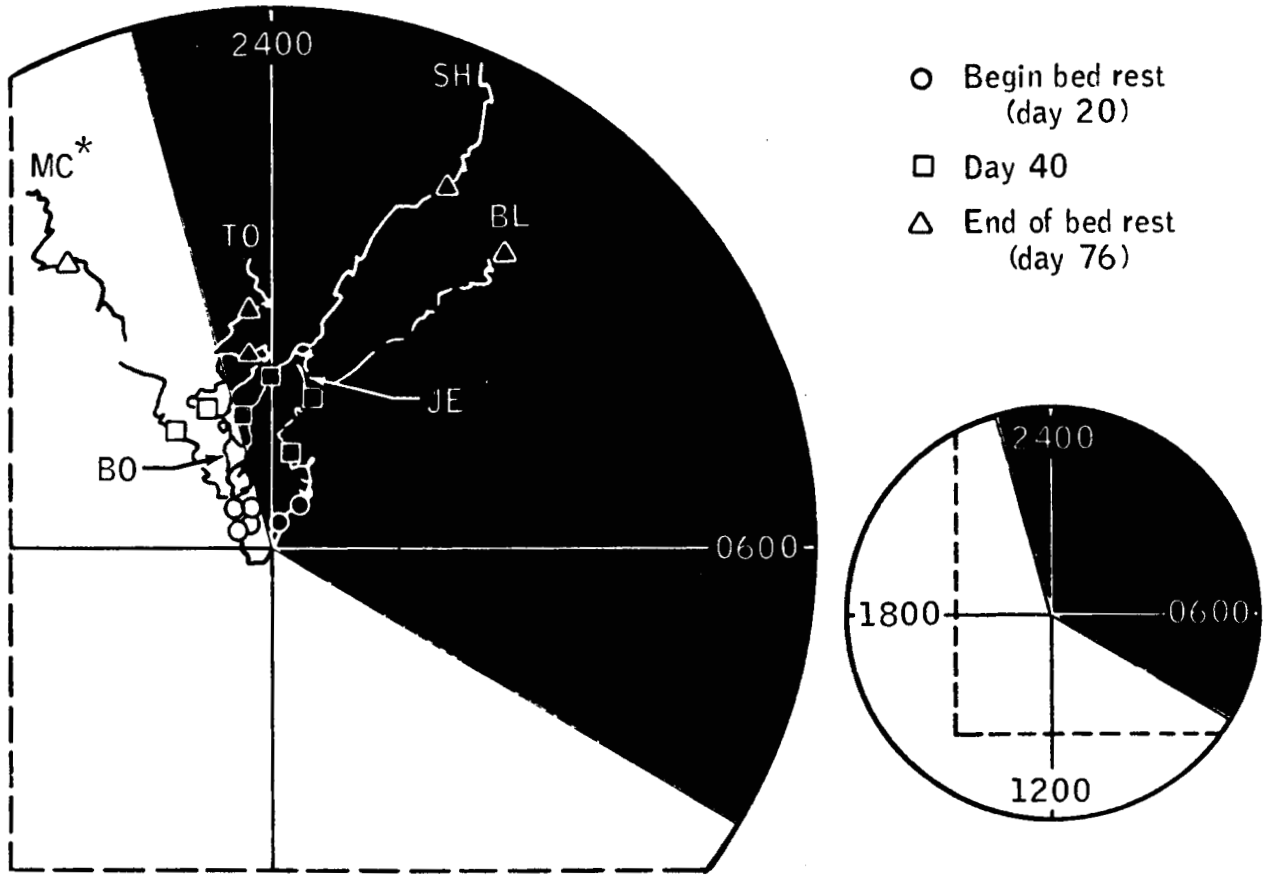


Figure 3-1.- Summation dial showing successive addition of body temperature daily vectors in order of day for subject 6A.



*MC = subject designation

Figure 3-2.- Body temperature summation dials for the ambulatory subjects.

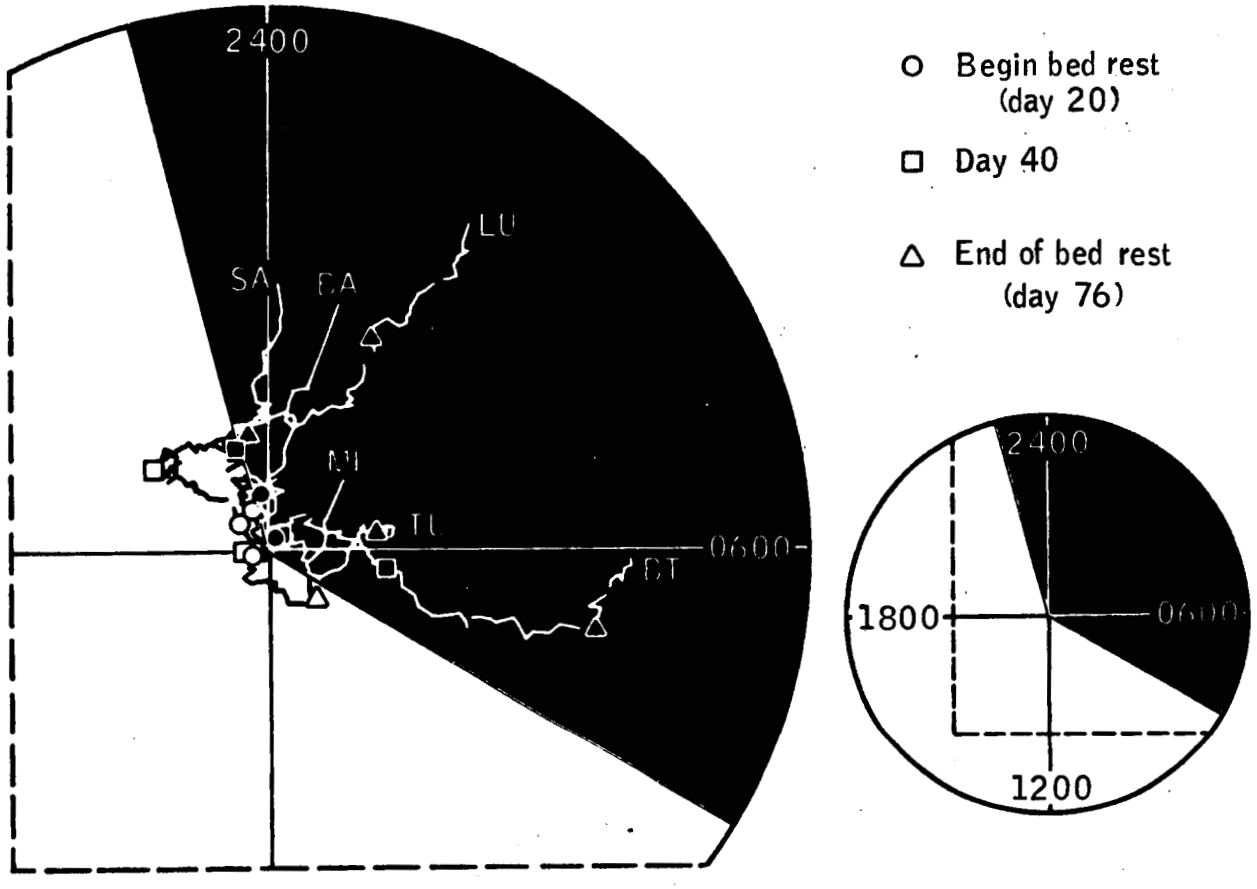


Figure 3-3.- Body temperature summation dials for the bed-rest subjects.

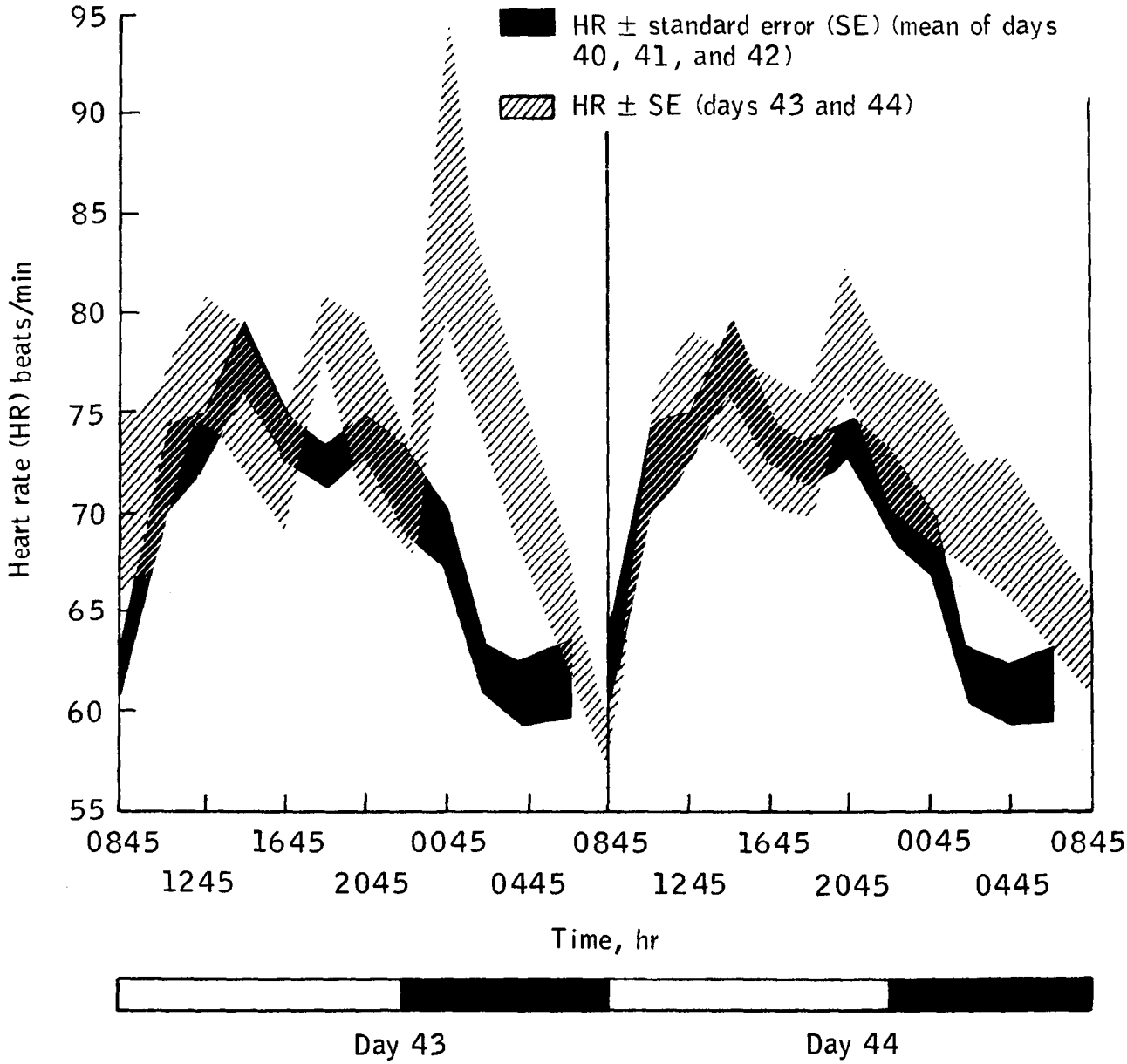


Figure 3-4.- Mean heart rate of four subjects on days 23 and 24 during bed rest (days 43 and 44 of experiment) compared to that of the same subjects during the three previous days. (Bar at bottom shows light-dark regime.)

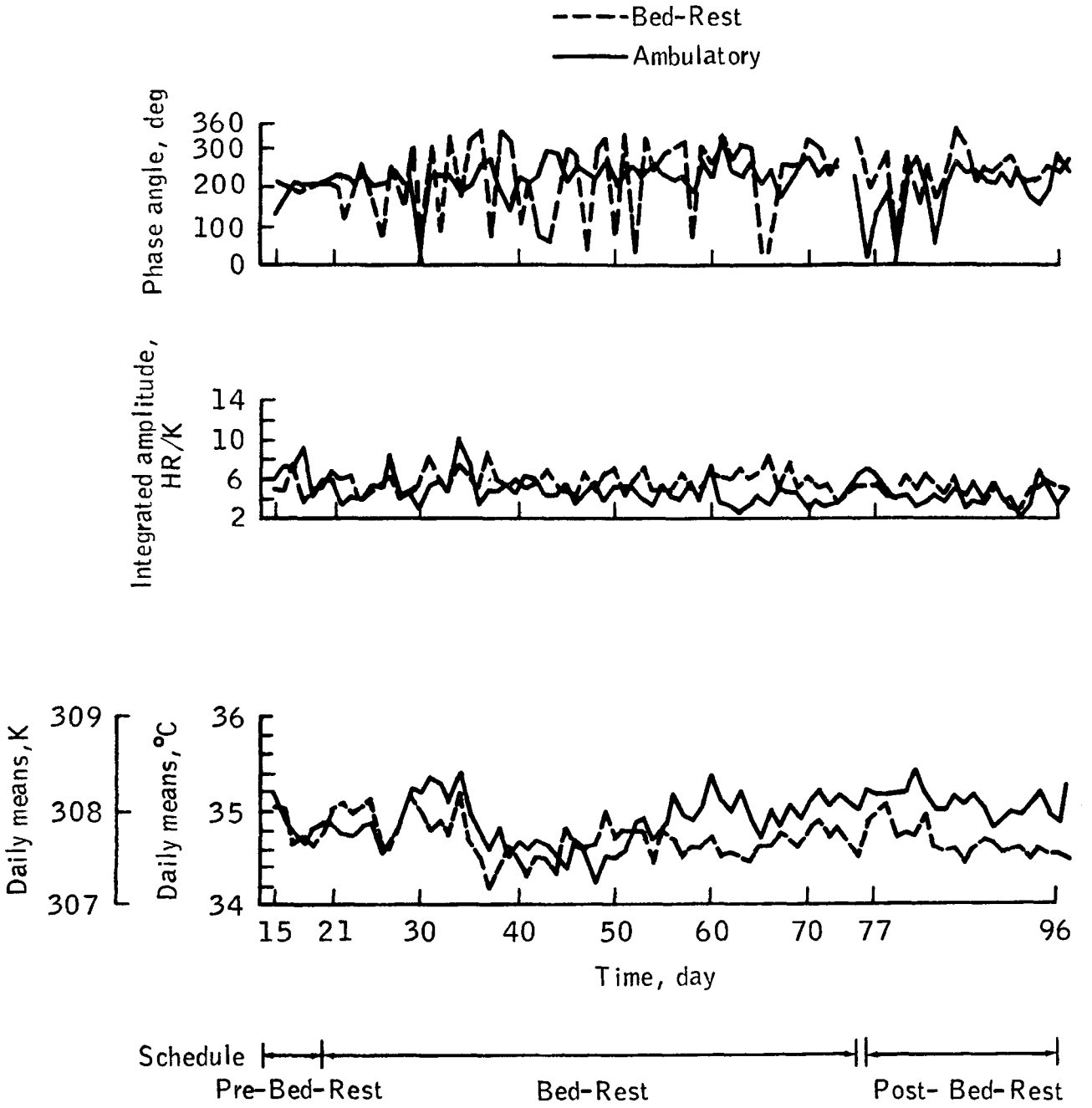


Figure 3-5.- A comparison of daily phase angle, integrated amplitude, and daily means of body temperature in ambulatory and bed-rest subjects.

4. STRESS-INDUCED CHANGES IN CORTICOSTEROID METABOLISM IN MAN

By Martha M. Tacker, Ph. D.

Secretion of glucocorticoids by the adrenal cortex is essential to man's ability to meet and survive a stressful situation. This secretion has been one of the physiological parameters used to evaluate an individual's response to stress and is currently determined by measuring plasma or urinary concentrations of cortisol, the principal glucocorticoid secreted by man. However, these concentrations are influenced by several factors other than adrenal secretion (e.g., plasma protein binding, distribution, catabolism, and excretion); and changes in these factors, as by stress, will alter cortisol concentration independently of adrenal secretion. If these changes are not recognized, the cortisol concentrations will not accurately reflect adrenal secretion, and comparisons between the stressed and nonstressed situations may be incorrect.

There has been a recurring difficulty in correlating and explaining cortisol concentrations measured in various NASA-supported studies. In postflight samples from Apollo crewmen, the low plasma-cortisol concentrations indicated decreased adrenal secretion of cortisol, whereas the relatively high urinary-cortisol levels indicated increased adrenal secretion. In prolonged bed-rest studies, the plasma-cortisol concentrations did not correlate well with the fluctuations found in the plasma adrenocorticotropic hormone (ACTH) levels. (Adrenocorticotropic hormone is the principal stimulus to glucocorticoid secretion.)

The stress of space flight possibly exceeds the capability of the adrenal to meet this stress, or it taxes the adrenal to such a point that any additional stress, such as trauma, could not be met adequately. Indeed, these unexpected cortisol concentrations may be reflecting such a situation. However, these concentrations could also be a result of stress-induced changes in the nonsecretory factors; in which case, the use of cortisol concentrations as an index of adrenal secretion would have to be modified. In either case, if cortisol concentrations are to be used as an index of the stress response, it is imperative to know how stress alters the contribution of any of the nonsecretory factors controlling cortisol concentration.

In a simplified view, plasma concentrations of cortisol are controlled by input and output (by adrenal secretion (input) and metabolic clearance (output)). If the metabolic clearance rate remains unchanged, plasma concentration will be a function of adrenal secretion. In this case, changes in plasma concentration will be a good approximation of changes in adrenal secretion.

In addition to serving as an index of adrenal-secretory activity, measurement of plasma concentrations is of value in its own right. It is, after all, the cortisol in the blood that is available to the target tissues and, therefore, of physiologic interest.

Metabolic clearance is composed of several factors, most of which fall into one of three general compartments: distribution, catabolism, and excretion (fig. 4-1). These compartments are both expandable and contractable. Aspects of these compartments can either be dependent on or independent of adrenal secretion and/or plasma concentration. For example, the percentage of cortisol catabolized varies with plasma concentration of cortisol, whereas the amount of cortisol excreted can be altered by changes in kidney function independent of plasma levels of cortisol. Finally, these compartments may not just remove cortisol from the plasma, but in certain cases, they may return cortisol to the plasma. This could occur by tissues releasing cortisol (ref. 4-1) or by chemical conversion, for example, from cortisone.

When data, such as those in the NASA studies, cannot be readily interpreted and reconciled according to preexisting concepts, the assumptions underlying these concepts must be reexamined and tested. One can speculate about mechanisms that produce such "discrepancies;" and, indeed, altered roles of some nonsecretory factors have been suggested by some investigators. However, there has been surprisingly little work to substantiate these speculations, and no definitive study has been made on the effects of stress on the role of nonsecretory factors in control of plasma concentrations of cortisol.

The enigma of the corticosteroid data remains unsolved and demands attention for interpretation of data from future NASA studies. This becomes even more important with increases in the length of time the astronauts will stay in a weightless environment, such as in Skylab experiments. Evaluation of the roles of various factors controlling corticosteroid concentrations will not only benefit NASA but is imperative for appropriate interpretation of corticosteroid data in virtually all research in which pituitary-adrenal function is measured by plasma or urinary concentrations. These incentives, plus the fact that recent advances have produced the necessary technical capability, demand that such studies be initiated now.

The basic question is whether plasma cortisol is the most appropriate and most informative measurement of the stress response. Although the word "cortisol" has been used in this discussion, the analytical method used generally and also at NASA Lyndon B. Johnson Space Center (JSC) (the corticosteroid-binding-protein technique) actually measures a mixture of corticosteroids. This method is generally adequate for most clinical studies because cortisol is the principal corticosteroid secreted by man. However, the adrenal cortex does secrete a variety of biologically active steroids, and the role of stress on the relative secretions of each steroid has not been defined. Because the investigation outlined in the following paragraphs is designed to elucidate mechanisms and make interpretations directly related to pituitary-adrenal function, each of the various components needs to be separated and examined. Therefore, amounts of the individual corticosteroids secreted by the adrenal cortex must be measured; this calls for even more specificity than has generally been used to date. Specific measurements of amounts of corticosteroids secreted are now available by using either radioimmunoassays or by separating corticosteroids by column chromatography; quantitation is also available by using the binding-protein assay (refs. 4-2 and 4-3).

The relevance of such specific studies is supported by two recent reports. Adrenocorticotropic hormone administration (the principal physiologic stimulus for corticosteroid secretion) has been reported to increase secretion of cortisol much more than it does corticosterone (ref. 4-4). In contrast, analysis of pre- and post-surgery plasma samples revealed that corticosterone increased proportionately more than cortisol (ref. 4-5).

If plasma cortisol is found to be the best, easiest, and most direct measurement of adrenal-cortex function, the second question is, "is it necessary to modify ones interpretation of changes in plasma cortisol in various circumstances?" That is to say, "is a significant amount of the change in plasma concentration resulting from altered distribution, catabolism, or excretion so that plasma values are not an accurate reflection of adrenal-cortex secretory activity?" The studies outlined in the following paragraphs are designed to provide information basic to answering these questions.

The studies will use normal male volunteers in both stressed and unstressed situations. Blood will be sampled at designated intervals before, during, and after the stress; urine will be collected during appropriate intervals. Measurements will be made of concentrations of cortisol, corticosterone, cortisone, 11-deoxycortisol (Compound S), and increases in deoxycorticosterone. Inclusion of 11-deoxycortisol and deoxycorticosterone is desirable because of their mineralocorticoid activity. If certain stresses cause significant increases in secretion of mineralocorticoids, this information would be valuable in explaining changes in fluid and electrolyte metabolism such as those that have been observed in the Apollo flights. Plasma ACTH measurements will be made at Baylor College of Medicine, Houston, Texas.

The studies currently planned are designed to follow plasma and urinary corticosteroid concentrations, urinary metabolites of the corticosteroids, and plasma ACTH. The effect of stress on the ratio of cortisol bound to the transport protein and cortisol free in the plasma will be measured. By using radioisotope techniques, plasma concentrations will be correlated with adrenal secretion of cortisol, and changes in distribution will be measured, initially, by measuring shifts in the amounts found in plasma and blood cells. Removal of cortisol from the plasma will be correlated with appearance in the urine; time relationships and the role of changes in kidney function will be evaluated. Changes in catabolism will be measured by changes in the urinary metabolites.

The time relationships of the adrenal response to stress are of physiologic interest in view of the recent reports of a diphasic or triphasic response in stressed animals (refs. 4-6 and 4-7). This information is of practical value regarding time of sampling and interpretation of values of plasma concentrations subsequent to stress.

An examination has been proposed to determine the effects of muscular exercise on the metabolism (secretion, binding, catabolism, and excretion) of corticosteroids in man by using a homogeneous population of trained subjects and rigidly controlled exercise. Subsequent studies would investigate the effect of an altered nutritional status (for example, potassium deficiency) as well as various stresses such as heat or psychic.

This study will provide a better understanding of the factors controlling the circulating concentrations of corticosteroids as well as indicate how well urinary and plasma cortisol concentrations can be correlated under conditions of stress. Investigators will be able to decide if plasma and urinary concentrations of cortisol are the appropriate and most informative measurements to reflect adrenal-cortical secretory activity. Furthermore, this study will document altered secretion of various types of corticosteroids that play a significant role in fluid and electrolyte metabolism; lastly, it will reveal the similarities and differences in the effects of different types of stress.

In addition to providing practical information for interpretation of data from NASA-sponsored and other stress studies (including space flights), these studies are of interest from other physiological viewpoints. For example, the proposed investigation will serve as a definitive study of the effects of exercise on corticosteroid metabolism in man, an area that remains controversial at this time. Furthermore, a better understanding of all the influences on the concentration of unbound cortisol in plasma, the biologically active fraction, will be invaluable in further elucidating the physiology of glucocorticoids in man.

REFERENCES

4-1. Courtney, G. A.; and Marotta, S. F.: Adrenocortical Steroids During Acute Exposure to Environmental Stresses: II. Uptake and Release of Infused Cortisol by the Hind Limb of Dogs. Aerospace Med., vol. 43, no. 1, Jan. 1972, pp. 52-55

4-2. Brown, Ronald D.; and Strott, Charles A.: Plasma Deoxycorticosterone in Man. J. Clin. Endocrin. Metab., vol. 32, 1971, pp. 744-750.

4-3. Newsome, H. H., Jr.; Clements, A. S.; and Borum, E. H.: The Simultaneous Assay of Cortisol, Corticosterone, 11-Deoxycortisol, and Cortisone in Human Plasma. J. Clin. Endocrin. Metab., vol. 34, no. 3, Feb. 1972, pp. 473-483.

4-4. Kolanowski, J.; Pizarro, M. A.; and Crabbe, J.: Changes in Adrenocortical Response to Corticotropin (ACTH) Administered Repeatedly in Man. Endocrine Society Mtg., 1972, p. 115.

4-5. Hamanaka, Y.; Manabe, H.; Tanaka, H.; Monden, Y.; et al.: Effects of Surgery on Plasma Levels of Cortisol, Corticosterone and Non-Protein-Bound Cortisol. Acta Endocrin., vol. 64, 1970, pp. 439-451.

4-6. Dallmen, M. F.; Jones, M. T.; Vernikos-Danellis, J.; and Ganong, W. F.: Corticosteroid Feedback Control of ACTH Secretion: Rapid Effects of Bilateral Adrenolectomy on Plasma ACTH in the Rat. Endocrinology, vol. 91, 1972, pp. 961-968.

4-7. Espiner, E. A.; Hart, D. S.; and Beaven, D. W.: Cortisol Secretion During Acute Stress and Response to Dexamethasone in Sheep with Adrenal Transplants. Endocrinology, vol. 90, 1972, pp. 1510-1514.

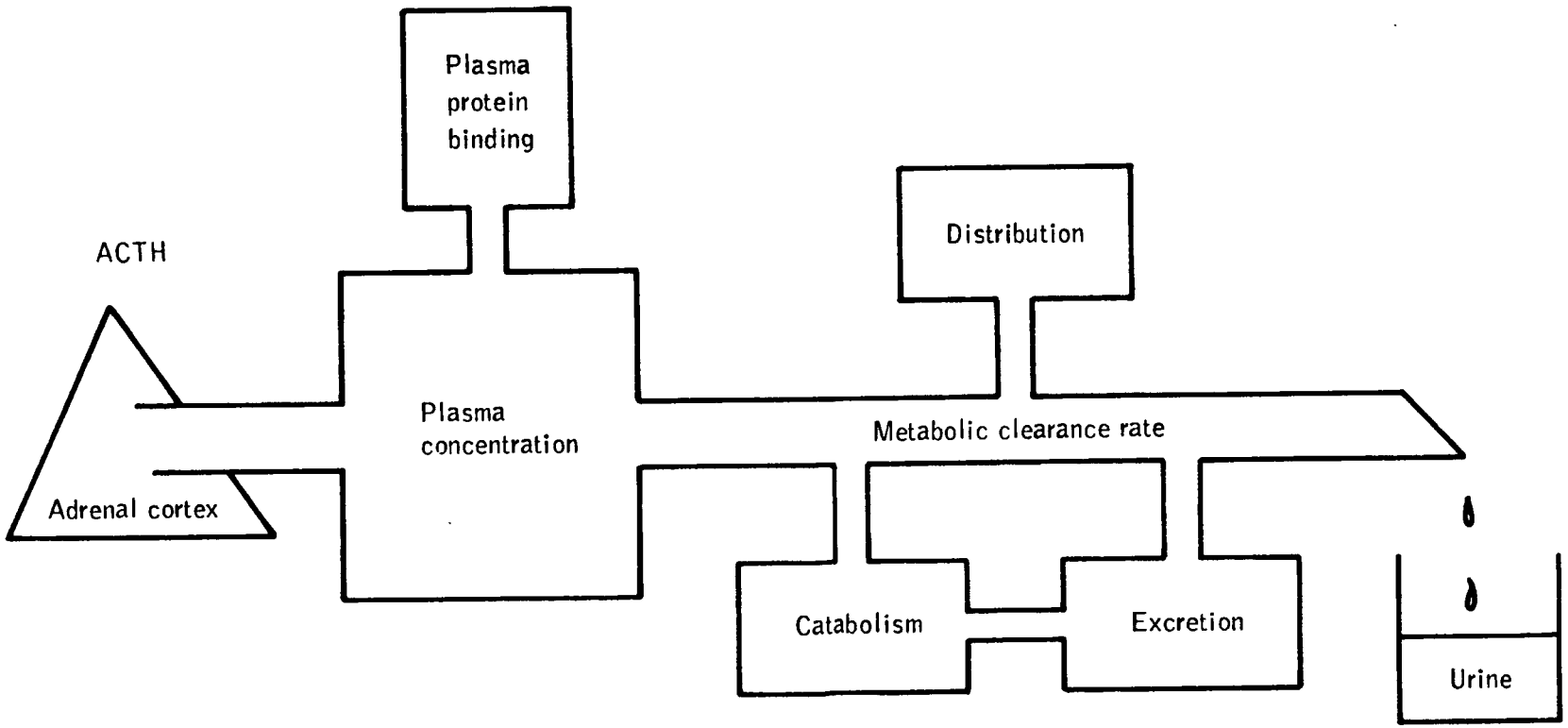


Figure 4-1.- Diagram illustrating factors of metabolic clearance.

5. PARATHYROID HORMONE AND VITAMIN D:

PRESENT STATUS OF PHYSIOLOGICAL STUDIES

By John T. Potts, Jr., M.D., and K. G. Swenson

PARATHYROID HORMONE

Rapid advancement in the knowledge regarding the chemical properties of parathyroid hormones has been achieved in the past 2 years. The complete amino acid sequence of the major form of the bovine hormone has been determined (refs. 5-1 and 5-2), and the structure of porcine parathyroid hormone has also been defined (ref. 5-3). Synthesis of peptides consisting of the amino terminal 34 residues of the bovine and porcine hormone and numerous shorter sequences and analogs of the amino terminal sequence of both molecules have permitted studies of the minimum structural features required for biological activity (fig. 5-1 and ref. 5-4).

The information concerning structure-activity relations of parathyroid hormone, which has resulted from these studies of the biological activity of synthetic fragments, has recently assumed great significance because of numerous reports indicating that the biosynthesis and metabolism of endogenous parathyroid hormone is quite complex. Multiple, chemically distinct forms of parathyroid hormone have been either identified or suspected to be present in the circulation (ref. 5-5). This complicates interpretation of radioimmunoassay studies in clinical disorders or in evaluating potential physiological changes, as in the studies relating to space flight.

Several years ago, it was demonstrated that plasma parathyroid hormone is immunologically distinct from hormone extracted from human adenomas. More recently (refs. 5-6 and 5-7), it was shown with in vitro studies that the immunoreactive parathyroid hormone, which accumulated in culture media when parathyroid glands were incubated in organ culture, was of smaller molecular size than the stored 84 amino acid hormone. This led to the suggestion that parathyroid hormone is cleaved in the parathyroid gland prior to secretion. This conclusion had the implication that the 84 amino acid peptide could not be the endogenous, active form of the hormone in vivo.

Laboratory findings (ref. 5-8) indicate that, in the peripheral circulation of both man and cow, the immunoreactive parathyroid hormone consists largely of a fragment(s) of the intact hormone. However, these studies have shown conclusively that this fragment(s) results from clea[illegible] the peripheral circulation and not from cleavage in the gland before release. The secreted hormone in man and cow is at least as large as the molecules extracted from glands.

Both the intact hormone and hormone fragment(s) are present in extremely low concentrations in plasma, so it has not yet been feasible to isolate the peptides in sufficient quantities for biological or chemical testing. Therefore, efforts in the authors' laboratory have concentrated on the development of radioimmunoassays in which antibodies have been developed or modified (ref. 5-9) to permit selective measurement of limited regions of the hormonal sequence. Because the minimum active sequence required for biological activity has been identified, immunochemical testing can be applied to determine whether any fragment in circulation has the structural features required for this activity. Thus far, studies with endogenous hormone have established, through gel filtration analysis of human peripheral plasma samples, that there is a large peak of immunoreactive material that elutes at a position corresponding to a molecular weight of approximately 7000. This fragment is detected using antisera that recognize antigenic determinants between residues 30 and 84, but not by antisera that recognize determinants amino terminal to position 30 (fig. 5-2(a)). Because this large fragment lacks a portion of the critical amino terminal sequence required for biological activity, it must be biologically inactive. Thus far, amino terminal reactivity has been detected only in the peak corresponding to intact hormone in peripheral human plasma (fig. 5-2(b), GP-1 preincubated with excess fragment 53 to 84).

Cleavage of the native peptide by an endopeptidase should result in formation of at least two fragments. With recognition of the large fragment, one might have expected to detect a second fragment of molecular weight 2500. However, no immunoreactive fragment has thus far been found that elutes later in the gel column effluent. Failure to detect such a small fragment with present antisera might mean only that the region of the molecule constituting the fragment is not recognized by the antisera. It may also mean, however, that the smaller fragment is destroyed at the time of cleavage or is cleared rapidly from the circulation in man.

However, in preliminary animal studies in which bovine hormone has been infused into the dog and cow and in studies of endogenous hormone in cows, a small fragment equivalent to a molecular weight of approximately 2500 as well as the large fragment of approximately 7000 molecular weight has been identified. This small fragment is detected by two amino terminal antisera that fail to react with the large fragment. These studies in animals have increased the suspicion that a smaller amino terminal fragment will ultimately be found in human blood.

Only when the exact site of cleavage of the secreted hormonal molecule is determined, can one assess the potential biological significance of the small amino fragment observed in dogs and cows and surmised by analogy to be present in man. The synthetic studies permit the conclusion that any fragment of bovine parathyroid hormone, in order to be biologically active, must consist of a continuous peptide sequence beginning with residue number 2, valine, and extending as far as residue number 27, lysine. As illustrated in figure 5-3, if the cleavage occurs "amino terminal to position 27," both fragments produced by cleavage would be biologically inactive (based on structure-activity considerations (fig. 5-1)). If cleavage occurs "carboxy terminal to position 27," the amino terminal fragment resulting from the cleavage might have the structural requirements to be biologically active.

Present investigations involving use of a series of antisera with more carefully defined recognition sites will help to identify more precisely the site of cleavage.

In addition to these hitherto unappreciated features of hormone metabolism, complexity has been uncovered with respect to the biosynthesis of the hormone. Biosynthesis of a precursor (proparathyroid hormone) to human and bovine parathyroid hormone has been demonstrated (refs. 5-10 to 5-13).

Therefore, it is now known that human and bovine parathyroid hormones undergo at least two specific cleavages from the point of initial cellular biosynthesis to their ultimate disappearance from the circulation (fig. 5-4). The first of these cleavages occurs in the cell when proparathyroid hormone (molecular weight approximately 11 500 daltons) is converted to parathyroid hormone (molecular weight 9500 daltons), the predominant species of the hormone that is secreted into the circulation. After secretion, the hormone rapidly undergoes a second cleavage in the periphery; a peak, eluting later than native hormone, corresponding to a fragment of molecular weight approximately 7000 daltons is detected in the general circulation. Obviously, these specific cleavage steps could serve as points of metabolic control regulating both the amount of biologically active hormone available for secretion as well as the concentration of hormonally active peptides circulating and interacting with receptors in bone and kidney.

At the present time, it can be concluded that immunoassays of parathyroid hormone must be interpreted cautiously in view of the complex nature of circulating immunoreactive hormone. On the other hand, it is clear that immunoreactive hormone concentration in blood does correlate with chronic changes in parathyroid gland secretory activity. One can state by analogy that the concentration of overall immunoreactive hormone in blood relates to parathyroid secretory rate in a manner similar to the relation between urinary 17-hydroxycorticoid excretion and cortisol secretory rate. In states of primary or secondary hyperparathyroidism, basal immunoreactive hormone concentrations are either absolutely increased or inappropriately high in relation to serum calcium. High, or at least readily detectable, hormone concentrations despite hypercalcemia are seen in primary hyperparathyroidism; hormone concentrations are undetectable in hypercalcemia due to nonparathyroid related diseases where the parathyroids are chronically suppressed (fig. 5-5).

In all Apollo missions, hormone concentrations have been within the normal range before and after space flight. The results suggest that adaptive increases in parathyroid secretory rate have not accompanied an exposure to weightlessness of not longer than 13 days.

VITAMIN D

It is now appreciated that vitamin D is stored in the body after formation in the skin or absorption from the diet and is converted to 25-OH vitamin D (25-OH D) by a specific hydroxylase in the liver (ref. 5-14).

Although this compound is active in vitro (while vitamin D itself is inactive), it has been discovered very recently that further hydroxylation of the vitamin occurs in the kidney before it can act on its target tissues. It is now known that 25-OH D is metabolized in the kidney to 1,25-$(OH)_2$ vitamin D (1,25-$(OH)_2$ D), 24,25-$(OH)_2$ vitamin D (24,25-$(OH)_2$ D), and other dihydroxy metabolites that have not yet been fully identified (ref. 5-15). It has been shown that 1,25-$(OH)_2$ D stimulates calcium transport by the intestine and mobilizes bone calcium in D-deficient rats. The physiologic role of a second dihydroxy metabolite, 24,25-$(OH)_2$ D, is unclear, although pharmacologic doses mobilize skeletal calcium in the D-deficient rat while showing only limited stimulation of intestinal calcium transport (ref. 5-16).

The metabolic activation of vitamin D appears to be a regulated process in both the liver and the kidney. In vitro and in vivo experiments with the rat have suggested product inhibition of the liver hydroxylase. Using the competitive binding assay for D and OH-D (using a specific D binding protein from rat serum), the authors found wide variations in the serum OH-D levels (tenfold or greater) in D-deficient rats with little or no change in serum calcium and, in contrast, small changes in OH-D level with large changes in serum calcium (ref. 5-17). Indeed, in these latter, clinically deficient animals with hypocalcemia, stunted growth, and abnormal bones, the OH-D levels were reduced by one-third to one-half but were detectable (1 ng/ml) for at least 4 to 5 weeks after the onset of clinical deficiency. Therefore, there does not seem to be any direct correlation between serum OH-D levels and the metabolic consequences of D deficiency, but further attempts to reconcile these findings with previous concepts are currently underway.

Applying these techniques directly to studies in man has been difficult because vitamin D_3 (resulting from photoactivation of precursor in the skin and the physiologic form in man) and vitamin D_2 (from dietary sources) are not equipotent in the assay system with the rat binding protein, although they are equipotent biologically. It was discovered, however, that the vitamin D binding protein in chick sera is relatively insensitive to vitamin D_2 and hydroxylated D_2 metabolites and might provide the basis for development of a selective assay for D_3 and its metabolites with the determination of total biologically active D (D_2 and D_3) by subtraction analysis. Although crystalline vitamin D_2 has been available, the lack of reliable hydroxylated D_2 metabolites or radioactive D_2 has impeded further development of these assays. Hydroxylated D_2 metabolites have been generated by feeding D-deficient rats large doses of vitamin D_2, with subsequent isolation of these metabolites from their blood. The authors have now purified the plasma extract free of other D metabolites by gel filtration

chromatography over Sephadex LH-20 and have approximately 100 grams of OH D_2 that is still contaminated with a large amount of non-D lipid. Further purification of this material is presently underway.

In related studies, it has been demonstrated that the D transport protein from rat plasma preferentially binds the 25 hydroxylated metabolites of vitamin D. This has made possible the development of an assay for 25-OH vitamin D directly from ethanol extracts of serum (eliminating cumbersome, time-consuming, preparative chromatographic separation of D and OH-D), thus facilitating the processing of many more samples much more rapidly (1 day compared to 10 days). Although preliminary studies have been undertaken in some selected human sera (where D deficiency or gross excess was suspected), most studies have focused on a rat model where the animals acted as their own controls (serial studies), as during pregnancy, lactation, and postlactation, or where the nature of the D intake was controlled (D_3 or OH D_3 fed to D-deficient animals). The preliminary results have challenged the concept of product inhibition at the liver hydroxylase.

The discovery that 1,25-$(OH)_2$ D and 24,25-$(OH)_2$ D are the tissue specific forms of vitamin D has resulted in a reassessment of the role of vitamin D in both normal and disordered calcium and skeletal homeostasis. Although extensive studies using primarily tracer kinetic analysis in vitro and in vivo have been done in the D-deficient rat, a number of questions regarding control mechanisms and metabolic determinants still remain even in this model. However, confirmation of these findings and their applicability to problems in man have been extremely limited, primarily because of the difficulties involved in tracer kinetic analysis in human subjects with endogenous vitamin D stores of uncertain amount and composition.

To determine the physiologic importance of the recent discovery that the kidney metabolizes 25-OH D to dihydroxy metabolites and to determine the role this may have in disordered calcium and skeletal homeostasis in man, further technological developments, most specifically the development of assays for the dihydroxy metabolites, are necessary. The authors have been able to show that 1,25-$(OH)_2$ D and 24,25-$(OH)_2$ D interact with the same binding protein used in the present assay (for D and 25-OH D) and to separate the dihydroxy metabolites from each other (as well as from D and 25-OH D) by chromatography over Sephadex LH-20. It is feasible to develop an assay for the dihydroxy metabolites with adequate sensitivity to detect these compounds in human plasma or serum. However, final development of these assays requires a reliable standard. (Synthesis of 1,25-$(OH)_2$ D_3 has been completed but final purification and quantitation is required, while 24,25-$(OH)_2$ D_3 has not yet been synthesized but only generated biologically.) The 1,25-$(OH)_2$ D_3 standard, which should be available within the next few months, will make possible the measurement not only of vitamin D itself but also of the monohydroxylated and dihydroxylated metabolites of D. Researchers may then assess the role of the renal hydroxylase(s) as well as that of liver hydroxylase in normal and abnormal vitamin D metabolism.

REFERENCES

5-1. Brewer, H. B., Jr.; and Ronan, R.: Bovine Parathyroid Hormone Amino Acid Sequence. Proc. Nat. Acad. Sci. U.S., vol. 67, no. 4, Dec. 1970, pp. 1862-1869.

5-2. Niall, H. D.; Keutmann, H. T.; Sauer, R.; Hogan, J. L.; et al.: The Amino Acid Sequence of Bovine Parathyroid Hormone I. Hoppe Seylers Zeitschrift Physiol. Chem., vol. 351, Dec. 1970, pp. 1586-1588.

5-3. Potts, J. T., Jr.; Keutmann, H. T.; Niall, H. D.; and Tregear, G. W.: Chemistry of Parathyroid Hormone and the Calcitonins. Vol. 29 of Vitamins and Hormones, Academic Press (New York), 1971, pp. 41-93.

5-4. Tregear, Geoffrey W.; Van Rietschoten, Jurphaas; Keutmann, Henry T.; Niall, Hugh D.; et al.: Bovine Parathyroid Hormone: Minimum Chain Length of Synthetic Peptide Required for Biological Acitivity. Endocrinology, vol. 93, no. 6, Dec. 1973, pp. 1349-1353.

5-5. Berson, Solomon A.; and Yalow, Rosalyn S.: Immunochemical Heterogeneity of Parathyroid Hormone in Plasma. J. Clin. Endocrin. Metab., vol. 28, July 1968, pp. 1037-1047.

5-6. Sherwood, Louis M.; Rodman, John S.; and Lundberg, Walter B: Evidence for a Precursor to Circulating Parathyroid Hormone. Proc. Nat. Acad. Sci. U.S., vol. 67, no. 3, Nov. 1970, pp. 1631-1638.

5-7. Arnaud, Claude D.; Sizemore, Glen W.; Oldham, Susan B.; Fischer, Jan A.; et al.: Human Parathyroid Hormone: Glandular and Secreted Molecular Species. Am. J. Med., vol. 50, no. 5, May 1971, pp. 630-638.

5-8. Habener, J. F.; Powell, D.; Murray, T. M.; Mayer, G. P.; and Potts, J. T., Jr.: Parathyroid Hormone: Secretion and Metabolism in vivo. Proc. Nat. Acad. Sci. U.S., vol. 68, no. 12, Dec. 1971, pp. 2986-2991.

5-9. Segre, Gino V.; Habener, Joel F.; Powell, David; Tregear, Geoffrey W.; and Potts, John T., Jr.: Parathyroid Hormone in Human Plasma, Immunochemical Analysis and Biological Implications. J. Clin. Invest., vol. 51, Dec. 1972, pp. 3163-3172.

5-10. Cohn, D. V.; MacGregor, R. R.; Chu, L. J. H.; and Hamilton, J. W.: Studies on the Biosynthesis in vivo of Parathyroid Hormone and Other Calcemic Polypeptides of the Parathyroid Gland. Proceedings of the Fourth Parathyroid Conference, R. V. Talmage and P. L. Munson, eds., Excerpta Medica (Amsterdam), 1972, p. 173.

5-11. Habener, J. F.; Kemper, B.; Potts, J. T., Jr.; and Rich, A.: Bovine Proparathyroid Hormone: Structural Analysis of Radioactive Peptides Formed by Limited Cleavage. Endocrinology, vol. 92, Jan. 1973, pp. 219-226.

5-12. Habener, Joel F.; Kemper, Byron; Potts, John T., Jr.; and Rich, Alexander: Proparathyroid Hormone: Biosynthesis by Human Parathyroid Adenomas. Science, vol. 178, no. 4061, Nov. 10, 1972, pp. 630-633.

5-13. Kemper, B.; Habener, J. F.; Potts, J. T., Jr.; and Rich, A.: Proparathyroid Hormone: Identification of a Biosynthetic Precursor to Parathyroid Hormone. Proc. Nat. Acad. Sci. U.S., vol. 69, no. 3, Mar. 1972, pp. 643-647.

5-14. Blunt, J. W.; DeLuca, H. F.; and Schnoes, H. K.: 25-Hydroxycholecalciferol: A Biologically Active Metabolite of Cholecalciferol. Chem. Commun., vol. 14, 1968, p. 801.

5-15. Holick, M. F.; Schnoes, H. K.; DeLuca, H. F.; Suda, T; and Cousins, J.: Isolation and Identification of 1,25-Dihydroxycholecalciferol. A Metabolite of Vitamin D Active in Intestine. Biochem., vol. 10, no. 14, July 1971, pp. 2799-2804.

5-16. DeLuca, H. F.: Vitamin D: New Horizons. Clin. Orthop., vol. 78, 1971, p. A-23.

5-17. Belsey, R.; DeLuca, H. F.; and Potts, J. T., Jr.: Competitive Binding Assay for Vitamin D and 25-OH Vitamin D. J. Clin. Endocrin. Metab., vol. 33, no. 3, Sept, 1971, pp. 554-557.

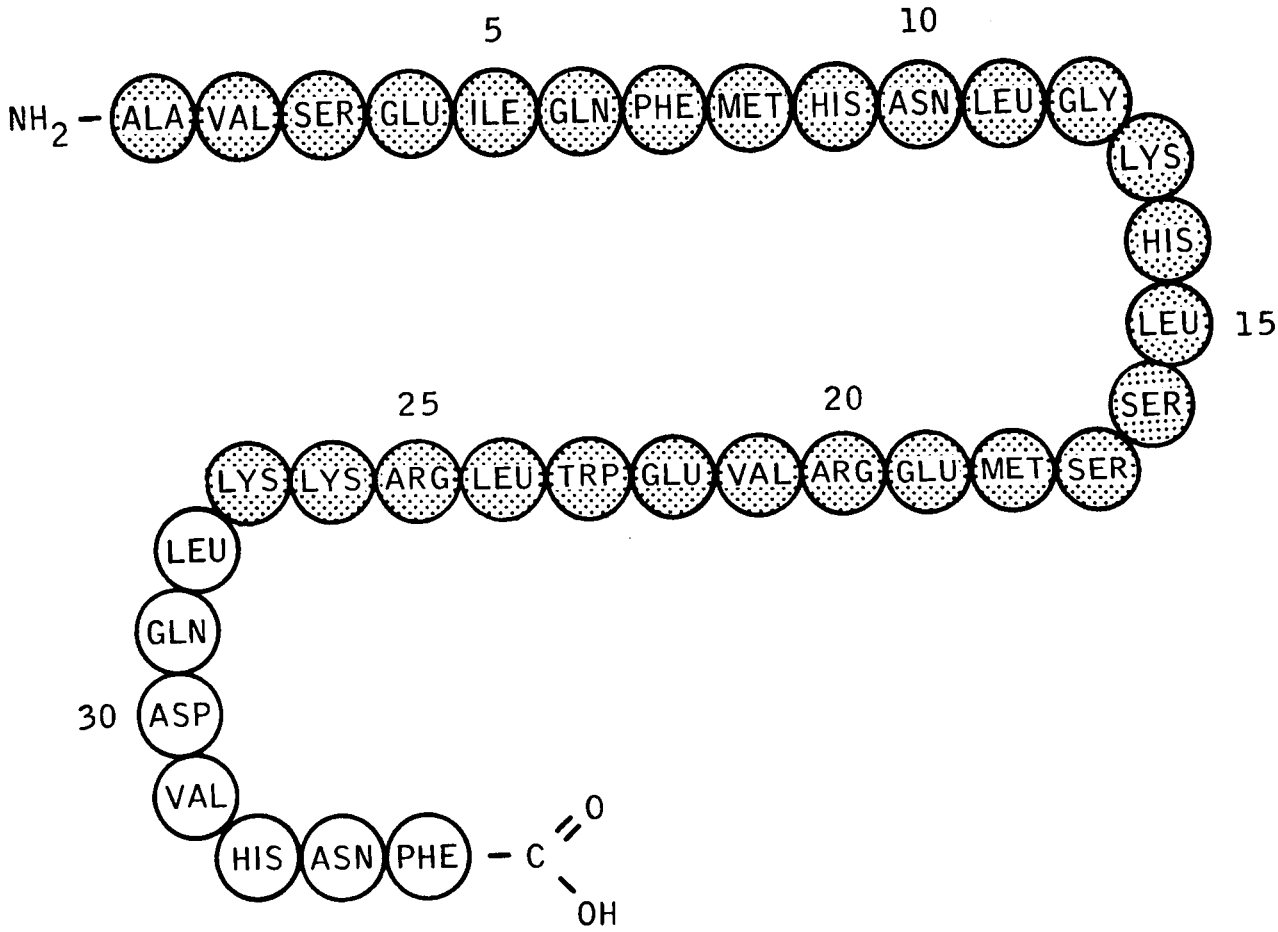
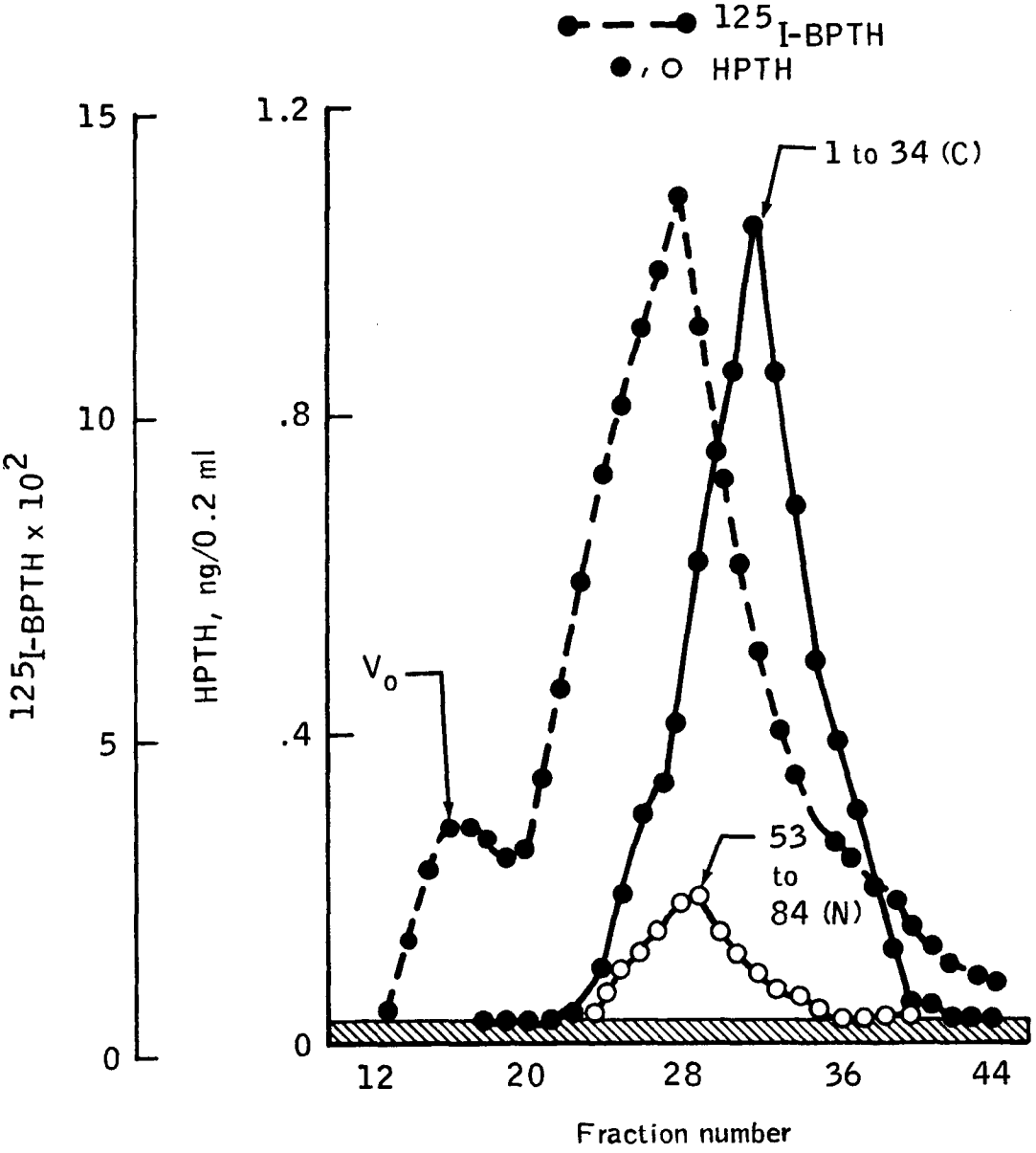
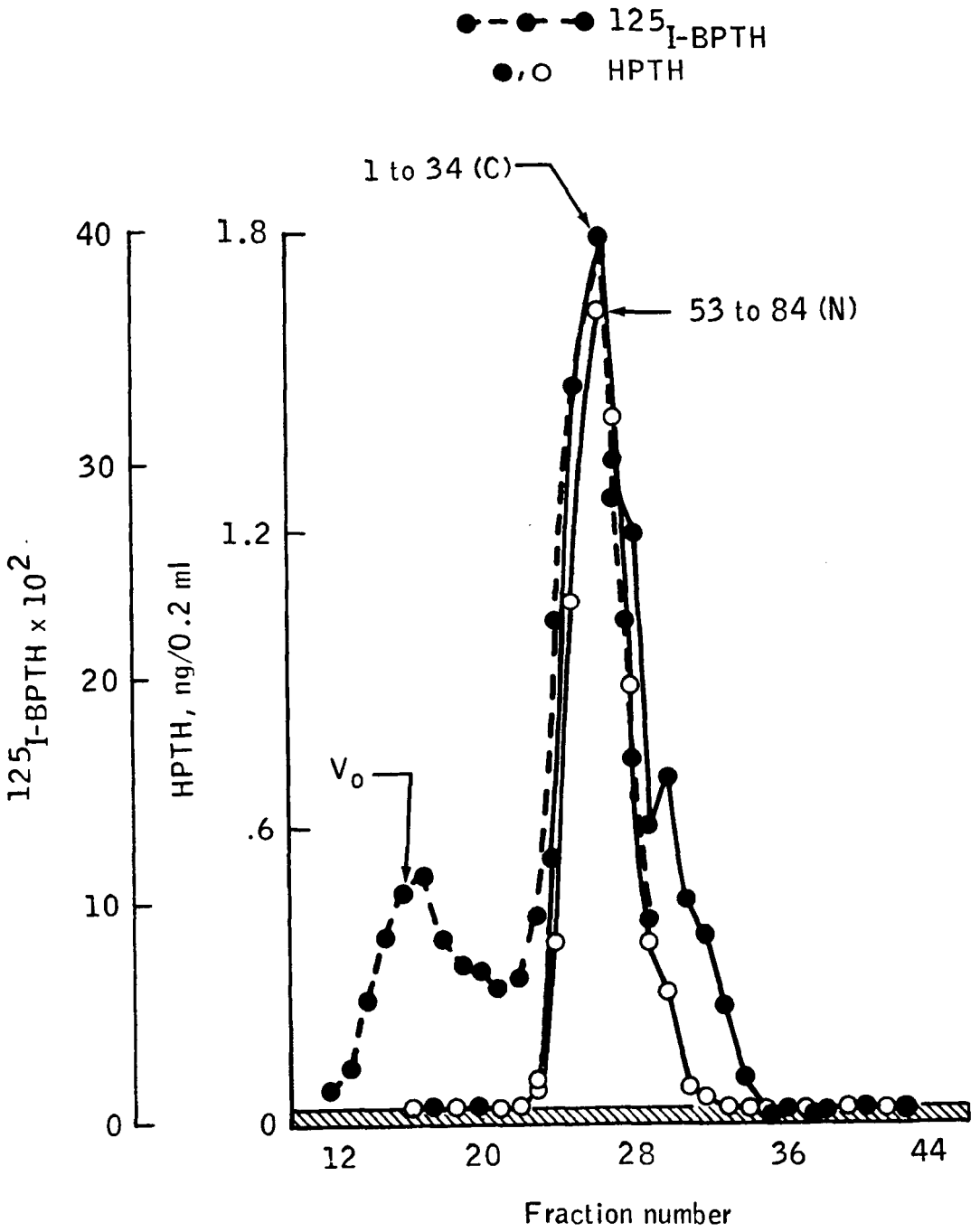


Figure 5-1.- The shaded area (1 to 27) of the first 34 residues of the bovine hormone indicates the minimal sequence required for biological activity.



(a) Peripheral vein.

Figure 5-2.- Comparison of immunoreactivity in fractions following gel filtration of human plasma samples on Bio-gel P-10. Samples from peripheral vein and parathyroid effluent vein, in assays using antisera GP-1 preincubated with excess concentration of fragments 1 to 34 (C-terminal assay) and GP-1 preincubated with excess concentration of fragments 53 to 84 (N-terminal assay). Concentration expressed in nanograms of human parathyroid hormone (HPTH)/0.2 ml fraction sample; the ^{125}I-bovine parathyroid hormone (BPTH) was co-chromatographed; V_o marks the void volume. The crosshatched area represents the sensitivity limits of the radioimmunoassays.



(b) Parathyroid effluent vein.

Figure 5-2.- Concluded.

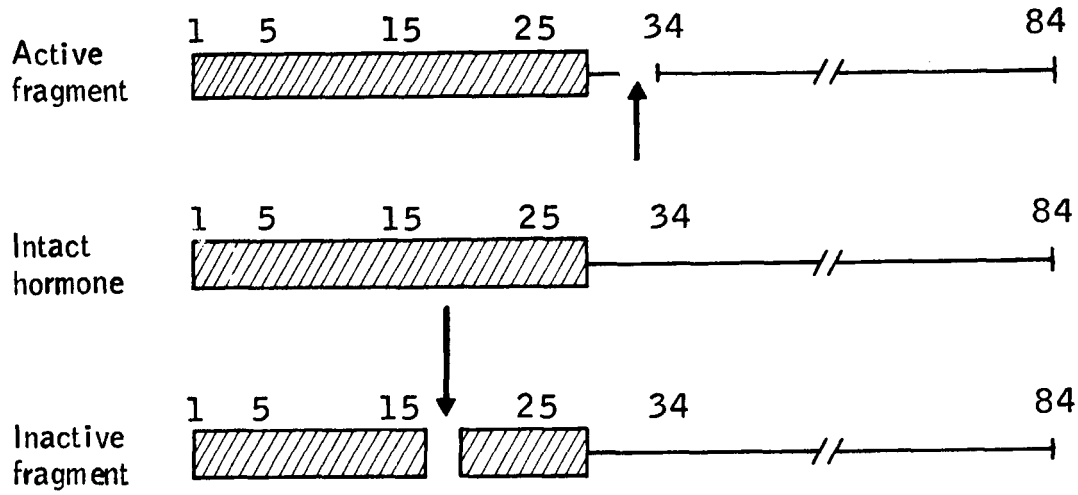


Figure 5-3.- Model of the two alternate metabolic cleavage sites of parathyroid hormone. The crosshatched area indicates the minimal sequence required for biological activity.

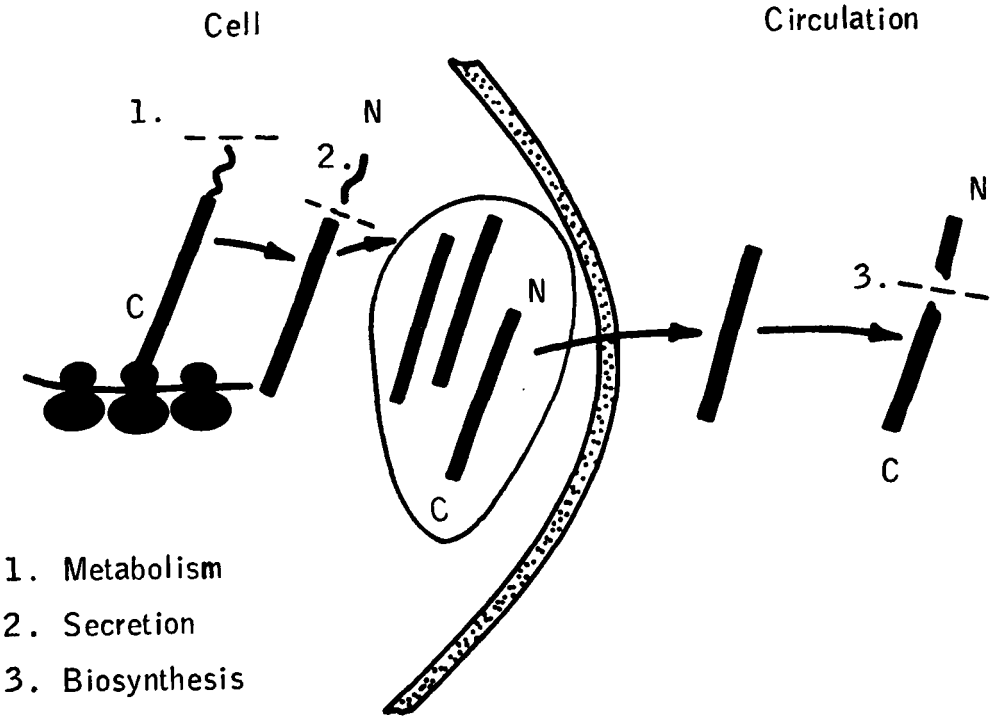


Figure 5-4.- Model of biosynthesis, secretion, and metabolism of parathyroid hormone in vivo.

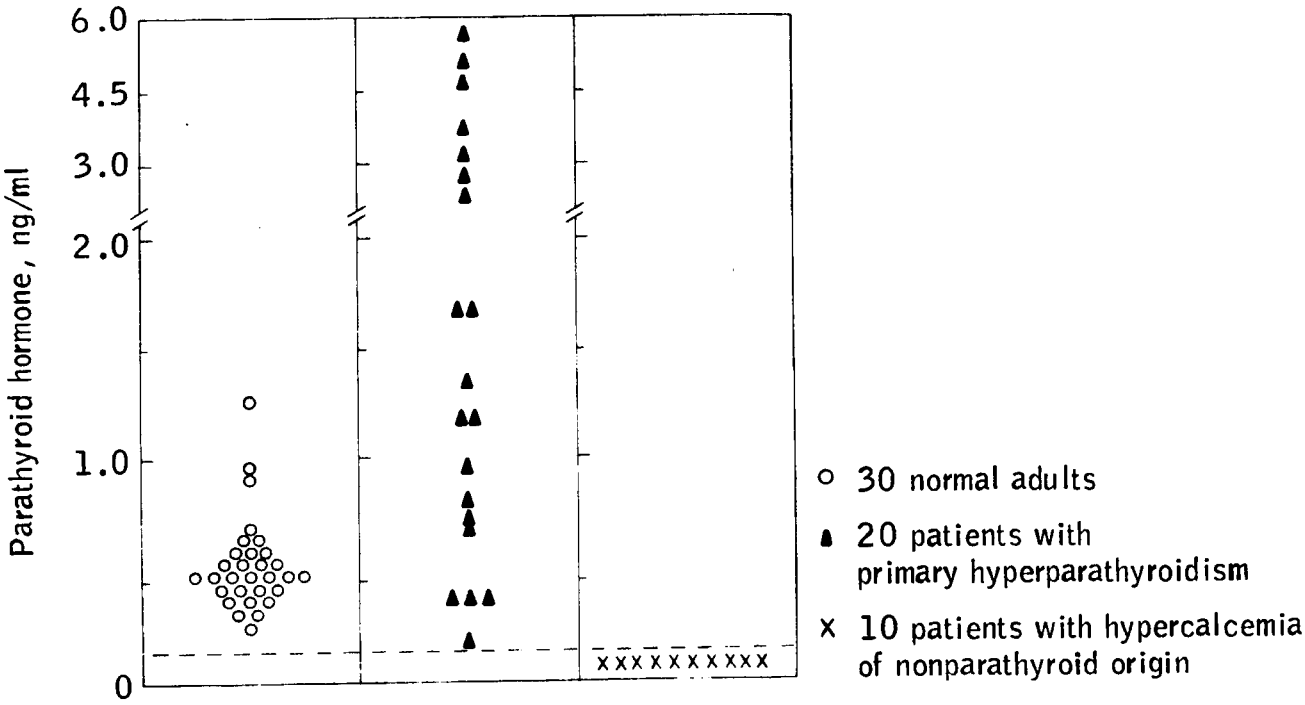


Figure 5-5.- Comparison of basal plasma parathyroid hormone concentrations in normal adults, patients with primary hyperparathyroidism, and patients with hypercalcemia of nonparathyroid origin. The dashed line indicates the usual minimum detection limit for plasma parathyroid hormone.

6. ANTAGONISTIC EFFECT OF LITHIUM ON ANTIDIURETIC HORMONE ACTION

By Myron Miller, M.D.

Studies dealing with the influence of various drugs on antidiuretic hormone (ADH) secretion or action have been made. In these studies, chlorpropamide (an oral sulfonylurea agent) has been shown to be an effective antidiuretic agent in the treatment of diabetes insipidus (DI) (ref. 6-1) and has been found to act both by stimulating ADH release from the neurohypophysis and by potentiating the action of small amounts of ADH at the renal tubule (refs. 6-2 and 6-3). More recently, the hypolipidemic agent clofibrate has been demonstrated to stimulate ADH release (ref. 6-4). As yet, no clinically usable drug is available that is capable of interfering with ADH release or of inhibiting ADH action on the kidney. Several reports have been written which demonstrate that lithium ion (Li^+) is capable of causing polyuria in animals and man (refs. 6-5 and 6-6). This report verifies the observation and provides data on the mechanism of Li^+ induced polyuria.

MATERIALS AND METHODS

Studies were made of normal rats and rats of the Brattleboro strain that were either heterozygous or homozygous for hereditary hypothalamic DI. The animals were housed in metabolic cages, and urine was collected at 24-hour intervals. After baseline collection periods, normal or heterozygous DI rats were given daily subcutaneous injections of lithium chloride (LiCl) solution, 0.3 to 0.4 meq/100 g body weight. Urine was collected for determination of volume, osmolality, ADH, sodium, potassium, and creatinine. After a control period, rats homozygous for DI were given subcutaneous vasopressin tannate in oil, 100 mU/100 g body weight. Half of this group also received simultaneous injections of LiCl, 0.3 meq/100 g body weight. Urine and pituitary ADH content were measured in one group of heterozygous DI rats before and after 2 days of dehydration. The ADH was measured by radioimmunoassay (refs. 6-7 and 6-8).

RESULTS

Normal rats given Li^+ 0.4 meq/100 g body weight developed polyuria and decrease in urine osmolality within 24 hours from initiation of treatment. This condition increased progressively so that by 5 days of treatment the mean urine volume increased from 7.1 to 38.2 ml/24 hr and mean urine osmolality decreased from 2734 to 648 mOsm/kg (fig. 6-1). A similar response occurred in

heterozygous DI rats given Li^+ 0.3 meq/100 g body weight (fig. 6-2). In these animals, urine volume rose progressively from a mean of 11.6 to 49.4 ml/24 hr, while mean urine osmolality fell from 1760 to 522 mOsm/kg. Urinary ADH excretion increased from 0.31 mU/24 hr before treatment to 0.82 mU/24 hr after 5 days of Li^+ treatment (fig. 6-3), thus indicating that the polyuria was not due to impaired ADH release. After 2 days of dehydration, urinary ADH excretion increased to 2.20 mU/24 hr in untreated animals and to 1.95 mU/24 hr in Li^+ treated rats, values not significantly different from each other (fig. 6-3). Four days of Li^+ treatment did not affect pituitary ADH content in normally hydrated rats and did not prevent the pituitary ADH depletion induced by 2 days of dehydration (fig. 6-4). These observations demonstrate that the Li^+ effect occurs without interference with ADH synthesis or release.

Injection of Li^+ 0.3 meq/100 g body weight blocked the antidiuretic effect of vasopressin given to rats homozygous for hereditary hypothalamic DI (fig. 6-5). The Li^+ treatment resulted in a significant decrease in the amount of injected ADH that was excreted in the urine (fig. 6-6).

DISCUSSION

The data presented here clearly demonstrate that Li^+ is capable of readily inducing polyuria both in normal rats and in rats with diminished neurohypophyseal ADH content. The polyuria occurs without associated decrease in urine ADH excretion and, in fact, there is a moderate increase in ADH excretion that is probably secondary to mild dehydration resulting from the marked polyuria. There is no apparent defect in neurohypophyseal ADH synthesis or release as a consequence of Li^+ treatment because pituitary ADH content is unaffected and 2 days of dehydration produce the expected increase in urine ADH excretion and decrease in posterior pituitary ADH content.

Lithium ion given to vasopressin-treated rats with severe DI can completely block the effect of the administered vasopressin. Therefore, it can be concluded that Li^+ induced polyuria results from an antagonistic effect of Li^+ on the action of ADH at the renal tubule level, and thus creates a state of nephrogenic DI. There appears to be no effect of Li^+ on ADH synthesis or release. From other data in published reports, it is suspected that Li^+ interferes with ADH stimulation of renal medullary adenyl cyclase and thus prevents formation of intracellular cyclic adenosinemonophosphate, the apparent mediator of ADH effect within the cell (refs. 6-9 and 6-10).

Lithium in the form of lithium carbonate is an approved drug currently used in the treatment of manic-depressive psychosis. The ability of Li^+ to cause ADH antagonism in the human similar to that produced in the rat has been

demonstrated by the observation that patients treated with lithium carbonate have developed a nephrogenic DI-like syndrome. Possibly, the diuretic effect of Li^+ may prove to be useful in the treatment of clinical states associated with excessive water retention.

REFERENCES

6-1. Miller, Myron; and Moses, Arnold M.: Mechanism of Chlorpropamide Action in Diabetes Insipidus. J. Clin. Endocrin., vol. 30, Apr. 1970, pp. 488-496.

6-2. Miller, Myron; and Moses, Arnold M.: Potentiation of Vasopressin Action by Chlorpropamide in vivo. Endocrinology, vol. 86, no. 5, May 1970, pp. 1024-1027.

6-3. Moses, Arnold M.; Numann, Patricia; and Miller, Myron: Mechanism of Chlorpropamide-Induced Antidiuresis in Man, Evidence for Release of ADH and Enhancement of Peripheral Action. Metab. (Clin. Exp.), vol. 22, no. 1, Jan. 1973, pp. 59-66.

6-4. Moses, Arnold M.; Howanitz, Joan; Van Gemert, Marcia; and Miller, Myron: Clofibrate-Induced Antidiuresis. J. Clin. Invest., vol. 52, no. 3, Mar. 1973, pp. 535-542.

6-5. Thomsen, K.: Lithium-Induced Polyuria in Rats. Int. Pharmacopsychiat., vol. 5, 1970, pp. 233-241.

6-6. Lee, Richard V.; Jampol, Lee M.; and Brown, W. Virgil: Nephrogenic Diabetes Insipidus and Lithium Intoxication - Complications of Lithium Carbonate Therapy. New Eng. J. Med., vol. 284, no. 2, 1971, pp. 93-94.

6-7. Miller, Myron; and Moses, Arnold M.: Radioimmunoassay of Vasopressin with a Comparison of Immunological and Biological Activity in the Rat Posterior Pituitary. Endocrinology, vol. 84, 1969, pp. 557-562.

6-8. Miller, Myron; and Moses, Arnold M.: Radioimmunoassay of Urinary Antidiuretic Hormone with Application to Study of the Brattleboro Rat. Endocrinology, vol. 88, 1971, pp. 1389-1396.

6-9. Dousa, Thomas; and Hechter, Oscar: The Effect of NaCl and LiCl on Vasopressin-Sensitive Adenyl Cyclase. Life Sci., vol. 9, no. 13, pt. 1, July 1970, pp. 765-770.

6-10. Geisler, A.; Wraae, O.; and Olesen, O. V.: Adenyl Cyclase Activity in Kidneys of Rats with Lithium-Induced Polyuria. Acta Pharmacol. et Toxicol., vol. 31, 1972, pp. 203-208.

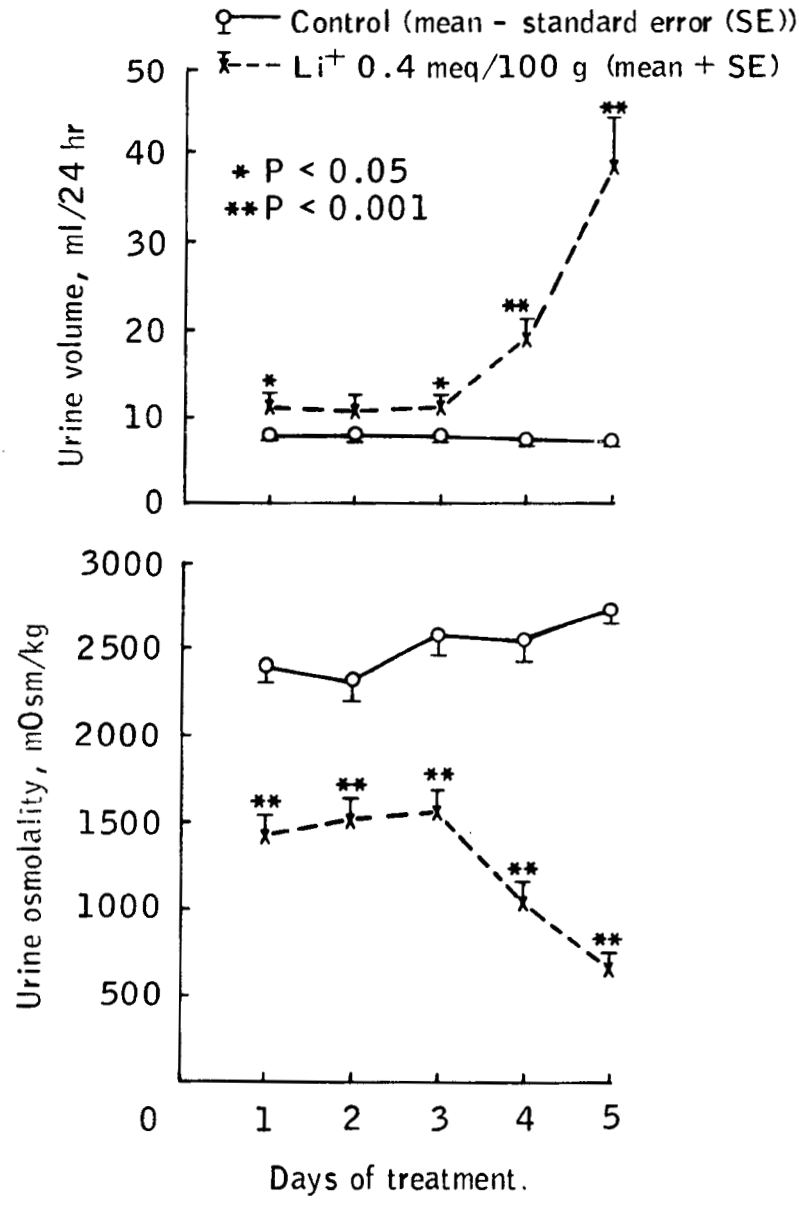


Figure 6-1.- Response of urine volume and osmolality to Li^+ injection in normal rats. Control animals received distilled water subcutaneously in a volume equivalent to that injected into Li^+ treated animals.

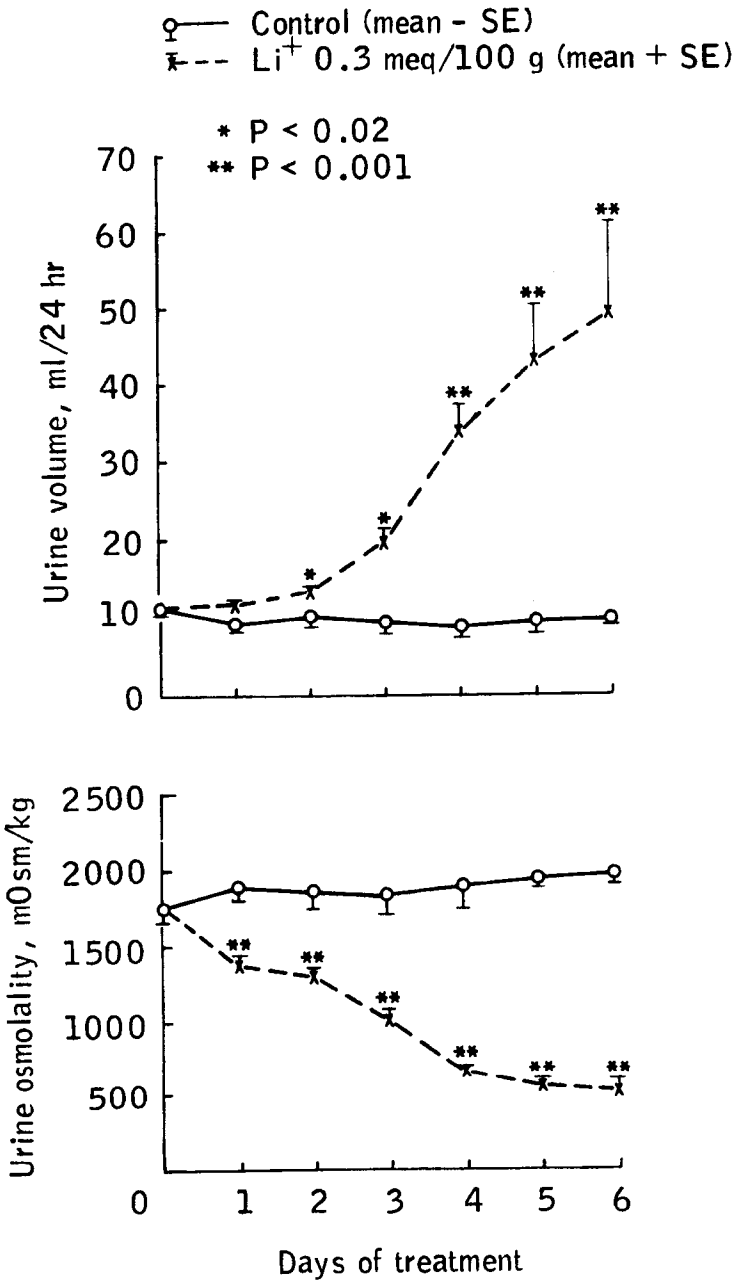


Figure 6-2.- Response of urine volume and osmolality to Li^+ injection in rats heterozygous for DI.

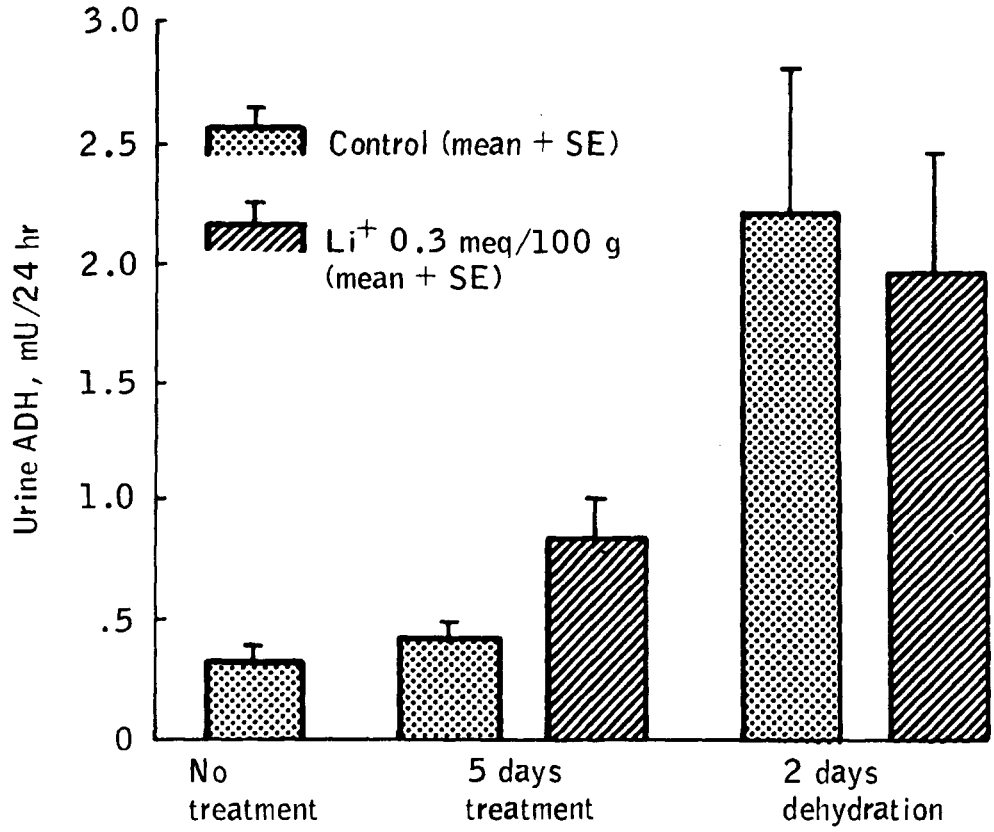


Figure 6-3.- Effect of Li^+ on urinary ADH excretion in heterozygous DI rats. Five days of treatment resulted in a moderate but statistically insignificant increase in ADH excretion. Urine ADH increased to the same extent following dehydration in untreated and Li^+ treated animals.

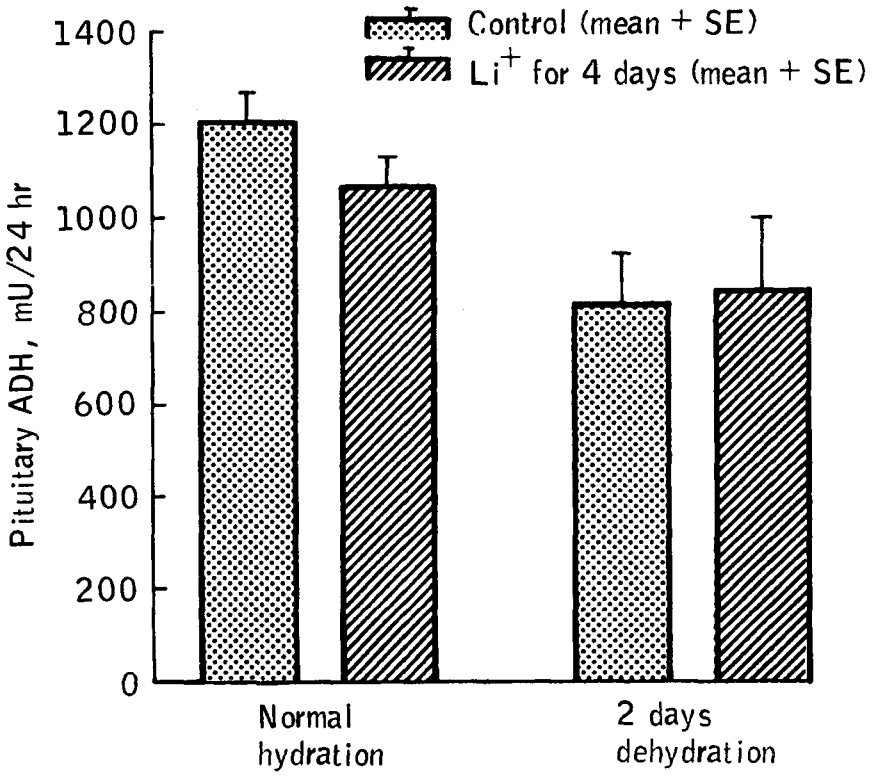


Figure 6-4.- Effect of Li^+ on posterior pituitary ADH content in heterozygous DI rats. Lithium ion treatment had no effect on the ADH content of normally hydrated rats or of rats dehydrated for 2 days.

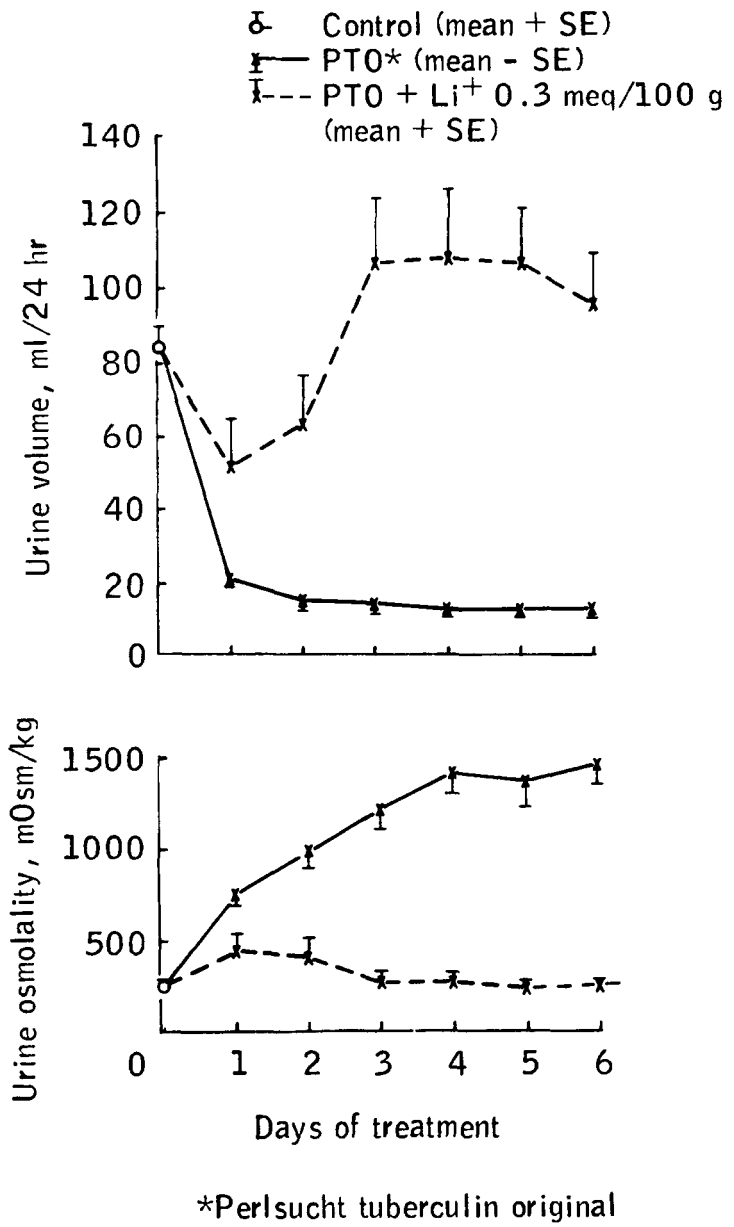


Figure 6-5.- Effect of Li^+ on vasopressin-induced antidiuresis in rats homozygous for DI. Lithium ion injection completely blocked the effect of vasopressin.

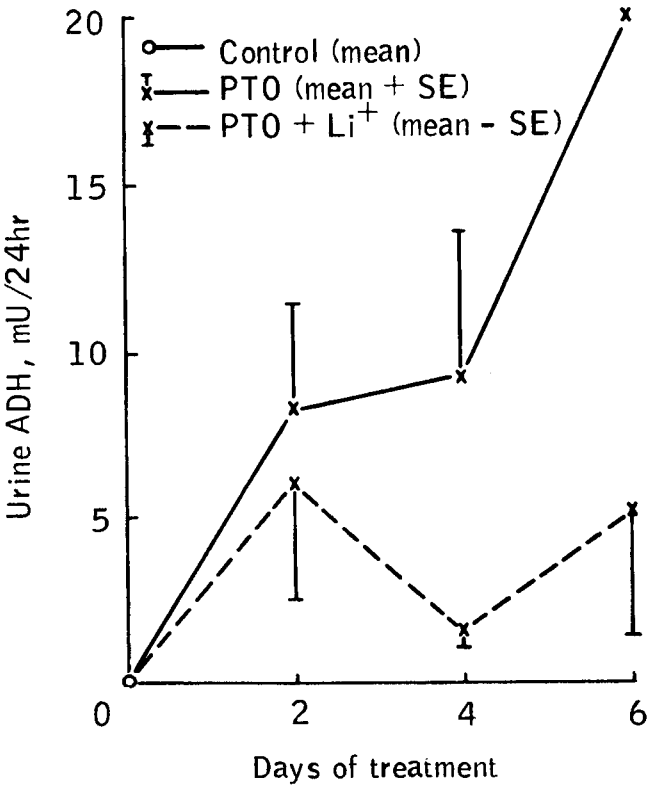


Figure 6-6.- Effect of Li^+ on urinary excretion of injected vasopressin in homozygous DI rats. Lithium ion significantly decreased the excretion of exogenous vasopressin.

7. SKYLAB BODY-FLUID VOLUMES STUDY

By Philip C. Johnson, M.D., and Carolyn S. Leach, Ph.D.

The body contains three measurable water-containing volumes: plasma volume, interstitial space, and intracellular water. The kidneys have a major role in maintaining each of the body-fluid compartments at adequate levels. Much of the renal regulation occurs in response to the water hormone (antidiuretic hormone) and electrolyte hormones (aldosterone, corticosterone, and hydrocortisone). Even slight changes in fluid volumes can trigger hormonal activity to which the kidney responds. Therefore, the study of these volumes should include hormone measurement; and, vice versa, the study of these hormones should include estimates of these volumes.

Extracellular fluid (ECF) consists of a vascular and nonvascular component (interstitial). The vascular portion (plasma volume) or ECF undergoes changes that seem to be related to the duration of the mission. The extravascular portion of the ECF might be expected to change, because it would seem logical that the upright posture on Earth would tend to cause an accumulation of interstitial fluid in the dependent portions of the body. Thus, interstitial fluid might have a labile portion similar to the labile portion of the plasma volume. The ECF measurements after prolonged bed rest seem to confirm this observation (ref. 7-1). However, the two Apollo missions in which ECF was measured suggest no significant change in the interstitial portion of the ECF. The mean-percent-volume change for the six crewmembers was -1.5 percent. When this was converted to percent change per ml/kg body weight, the value was +2.4 percent. This indicates that tissue loss rather than change in ECF was the cause of the body-weight loss.

Total body water (TBW) includes both extracellular and intracellular fluid. The extracellular portion of TBW is the ECF, and the intracellular portion is a measure of lean body mass. In the two Apollo missions in which TBW of the returning crewmembers was measured, the values indicate no significant change in intracellular fluid concentration. The results are consistent with tissue loss because the mean-percent TBW volume change was -2.1 percent, but when it was expressed as percent per ml/kg body weight, the mean was +0.7 percent.

Regulation of fluid volumes can occur primarily through hormones that affect water regulation or hormones that affect electrolyte regulation. No matter which hormone starts the process, both types of regulation eventually occur to effect an isotonic result. In weightlessness, the early change seems to be a fluid shift in the vascular volume that secondarily causes electrolyte effects by means of the adrenal hormones. Studies from the Apollo 15 mission indicate a significant loss of exchangeable potassium during that lunar landing mission. The most plausible explanation of a decrease in exchangeable potassium

is that weightlessness causes fluid shifts and losses that eventually require increased aldosterone production to counterbalance the effects of water loss. Under the influence of this hormone, potassium is lost and sodium is returned by the kidneys. The retention of sodium eventually increases plasma volume.

The concomitant loss of potassium and unchanged intracellular fluid found in the Apollo 15 mission suggest that the potassium loss represented both a loss of tissue cells, perhaps muscle cells, and a loss of potassium from the remaining cells. The cause of this tissue loss is undetermined. Certainly, lack of physical exercise would not explain it, because atrophy from disuse takes considerably longer than the 14 days of the longest mission to date.

Orbital flight produces changes in the vascular fluid compartments, and some of these changes seem related to the duration of the mission. Skylab, being the longest mission, will give investigators an opportunity to determine if the changes observed in shorter missions are progressive with time or merely time-limited changes associated with space flight.

The TBW, ECF, and total exchangeable-potassium methods that have been developed during the Apollo Program and that will be used for the Skylab studies have already been described.

Body-fluid spaces can be measured by various chemical and radionuclide techniques. Radionuclide methods were chosen because they do not require injection of large volumes of dyes or other substances that might cause changes in the systems or cause allergic reactions. Additionally, radionuclide procedures allow the use of smaller amounts of blood and urine. All radionuclide studies cause radiation exposure to the subject; the total body exposure that will result from the Skylab radionuclide studies is shown in table 7-I. This table also includes the radionuclides that will be used to determine blood volume and red-cell production and life span. These levels allow investigators to keep the total body radiation within the limits required for occupational exposure. Actual exposure of the crewmembers to date (from all sources including orbital and lunar missions, medical X-ray, and radionuclide studies) has been only a small fraction of the exposure allowed for continuous total body occupational exposure. Orbital missions, such as Skylab, will not be associated with significant radiation exposure unless unprogramed time is spent in the Van Allen belts and particularly in the South Atlantic anomaly. Considering this, there seems no chance that the dosage limits of the National Academy of Science will be approached in Skylab.

REFERENCE

7-1. Hyatt, Kenneth H.; Smith, William M.; Vogel, John M.; Sullivan, Robert W.; et al.: A Study of the Role of Extravascular Dehydration in the Production of Cardiovascular Deconditioning by Simulated Weightlessness (Bedrest). Pts. I and II, NASA CR-114808 and NASA CR-114809, 1970.

TABLE 7-I.- TOTAL BODY EXPOSURE TO RESULT FROM SKYLAB RADIONUCLIDE STUDIES

Nuclide	Physical form	Critical organ		Total body, rem/μCi	Total, μCi[a]	Total body, rem
		Organ	Exposure, rem/μCi			
Iodine-125	Albumin	Thyroid	0.0625 to 0.1875	0.00050	6	0.0030
Chromium-51	Chromate	Lung	.0020	.00036	75	.0270
Sulfur-35	Sulfate	Total body	.00009	.00009	75	.0068
Hydrogen-3	Water	Total body	.00017	.00017	75	.0128
Potassium-42	Chloride	Muscle	.00134	.00086	150	.1290
Iron-59	Chloride	Spleen	.1400	.03500	2	.0700
Carbon-14	Glycine	Bone	.00032	.00072	50	.0360
					Total	[b]0.2846

[a]Total for determinations made 21 days before lift-off, on recovery day, and 14 days after recovery.

[b]Maximum permissible total occupational body exposure is 1.25 rems/quarter. This can be increased to 3.0 rems/quarter if total for the year is less than 5.0 rems.

8. RECENT STUDIES ON DAILY RHYTHMIC CHANGES IN SEROTONIN CONTENT IN AREAS OF THE MOUSE BRAIN AND NOREPINEPHRINE CONTENT IN AREAS OF THE HAMSTER BRAIN

By William W. Morgan, Ph. D.

INTRODUCTION

The presence of diurnal rhythmic changes in the content of biogenic amines in the brains of rodents is well established. Diurnal changes in serotonin content (refs. 8-1 to 8-10), norepinephrine content and synthesis (refs. 8-3 to 8-5 and 8-10 to 8-16), dopamine content (refs. 8-5 and 8-15), and acetylcholine content (ref. 8-17) have been reported. Although the physiological significance of these phenomena is poorly understood, daily changes in some of the biogenic amines have been correlated with similar changes in other functional parameters. Friedman and Walker (ref. 8-3) have reported positive correlations between diurnal changes in rectal temperature and motor activity and the daily rhythm in norepinephrine content in rat brains. These same researchers found that the highest levels of brain serotonin during the day were correlated with the rest and sleep phases of the daily activity cycle. Schreiber and Schlesinger (ref. 8-18) found an inverse correlation between circadian variations in serotonin content and susceptibility to audiogenically induced seizures in DBA-2J mice. Scapagnini et al. (ref. 8-9) found a positive correlation between circadian changes in serotonin content in certain regions of the rat brain and similar daily changes in plasma corticosterone levels. After dampening of the serotonin rhythm by para-chlorophenylalanine (PCPA), these researchers found no daily changes in plasma corticosterone (ref. 8-9).

Although the presence of diurnal changes in the content of some of the biogenic amines is well documented and some evidence is available to suggest that they may have physiological significance, very little is known about the factors that produce or regulate these daily rhythmic changes in the content of the brain amines.

This report summarizes the results of recent experiments performed in the author's laboratory to investigate the factors involved in the production and regulation of daily rhythm changes in serotonin content in the mouse brain and similar changes in norepinephrine content in the hamster brain.

METHODS

The first series of experiments was undertaken to determine the nature of the daily rhythms in serotonin content in the cerebral cortex and the brainstem of mice. Five experiments of this type were performed. In each experiment, 36 adult male Balb/C or AJAX mice were housed in groups of six and maintained on a schedule of 12 hours of light and 12 hours of dark (12:12 LD), with the lights on from 0900 to 2100 daily. (All times are central standard time.) After 3 weeks, the animals were sacrificed in groups of six at 4-hour intervals over a 24-hour period. The first sacrifice was performed at 0800 and the last at 0400. Sacrifices at 4-hour intervals provided a good resolution of the serotonin rhythm without disturbance to the remaining animals by excessive entrance into the animal room. Sacrifices during the dark phase were performed with illumination provided by a red photographic safety light (Wratten 1 filter).

The animals were sacrificed by rapid decapitation. The skull of each animal was opened and the brain was removed. The cerebellum was discarded and the brainstem and cerebral cortex were dissected as described in reference 8-12. The dissected brain portions were weighed and put into cold (277 K (4° C)) acidified butanol for homogenization. Serotonin and norepinephrine were extracted by using the method of Maickel et al. (ref. 8-19). The 5-hydroxyindoleacetic acid (5-HIAA) content was determined, after further extraction and quantitation of each brain homogenate, by using the method of Curzon and Green (ref. 8-20). Serotonin was quantitated by using the o-phthaldialdehyde method of Maickel et al. (ref. 8-19) as modified by Alpers and Himwich (ref. 8-21). Norepinephrine was quantitated by the method of Chang (ref. 8-22). The statistical significance of the changes in the content of serotonin, norepinephrine, or 5-HIAA with time of day were determined by the analysis of variance.

In another experiment, the role of feeding and of plasma tryptophan in the regulation of the daily rhythm in serotonin content in the mouse brain was investigated. For this experiment, 72 adult male AJAX mice were divided into two equal groups. These animals were housed and exposed to the 12:12 LD regime as previously described. The control animals had free access to food (Purina Lab Chow) and water. The experimental animals were given water ad libitum but were given food for only 4 hours daily, between 1000 and 1400. After 5 weeks, the mice were weighed and then sacrificed by decapitation in groups of 12 (six controls and six experimentals) at 4-hour intervals over a 24-hour period as previously described. Blood from the cervical wound was collected in a heparinized 9-milliliter test tube. The brain was removed and divided longitudinally into symmetrical right and left halves. The serotonin content was determined in one half as described earlier; tryptophan content in the other half of brain was determined by using the method of Denckla and Dewey (ref. 8-23). The blood was centrifuged and the tryptophan content was determined in the plasma fraction. The statistical significance of the differences in plasma tryptophan, brain tryptophan, or brain serotonin with changes in the time of day in both control and experimental animals was determined by the analysis of variance. Effects of the feeding regime on the phasing (clock hour of peaks and troughs) of the daily rhythms in each of the above parameters were

determined by comparing graphs of the rhythm for each parameter in control as opposed to experimental animals.

In a recent experiment, the turnover of serotonin was measured in AJAX mice at various times of the day to determine how changes in the rate of synthesis or degradation of serotonin might be related to diurnal changes in the serotonin content in the mouse brain. As before, 36 mice were housed six per cage and exposed to the 12:12 LD regime. At 0800, six mice were given an intraperitoneal injection of probenecid (200 mg/kg) to inhibit the active transport of 5-HIAA from the brain. The probenecid was dissolved in 1N sodium hydroxide and then buffered to pH 7.4 with 0.5M phosphate buffer. At the same time, six non-injected control mice were sacrificed. Their brains were dissected into brainstem and cerebral cortex portions, as previously described. Serotonin and 5-HIAA were extracted and quantitated in these brain areas. Two hours later (at 1000) the probenecid-treated animals were sacrificed and their brains were collected and analyzed in the same manner as the controls. The same procedure of injecting probenecid into animals and sacrificing of controls was repeated at 1600 and 0200. The animals treated with probenecid at these time periods were sacrificed at 1800 and 0400, respectively. The percent increase in 5-HIAA content over the 2-hour period in the probenecid-treated animals was determined by subtracting the mean level of 5-HIAA in the noninjected controls from the mean 5-HIAA level in the probenecid-treated animals sacrificed 2 hours later and the division of this value by the control 5-HIAA value. The statistical significance of the differences in the total 5-HIAA levels and the serotonin levels in the control and in the probenecid-treated animals at different times of the day was determined by the analysis of variance.

Experiments were also undertaken to investigate the nature of daily rhythmic changes in the levels of norepinephrine in the brainstem, the diencephalon, and the cerebral cortex of male golden hamsters. In each experiment, 6-week-old male golden hamsters (Lakeview, New Jersey) were housed six per cage and exposed to a 14:10 LD regime. The light phase was from 0600 to 2000 daily, and room temperature was maintained almost constant at 295 K (22° C). Food and water were available ad libitum.

In the first study, 24 hamsters were housed for a period of 3 months. On the day of sacrifice, the hamsters were sacrificed in groups of six at 0800, 1400, 2000, and 0100. Sacrifice of all animals in the respective groups was completed within 45 minutes. During the dark phase, animals were sacrificed under a dim red light; after sacrifice, the skull was opened immediately and the whole brain removed. The cerebellum, the pineal gland, and the olfactory bulbs were dissected and discarded. The brainstem was separated from the spinal cord by a transverse section just posterior to the fourth ventricle. The dissection of the brainstem was completed with a transverse section just anterior to the superior colliculi. The brain was inverted and the dissection of the diencephalon was begun by two sagittal cuts, one on each side of the hypothalamus, made slightly oblique to cut parallel to the internal capsule. A transverse cut through the region of the anterior commissure completed the dissection of the diencephalon. The remainder of the brain was considered collectively as the cerebral cortex. Norepinephrine content in each brain area was extracted by using the method of Maickel et al. (ref. 8-19) and quantitated by the method of Chang (ref. 8-22).

In a second study on the daily norepinephrine rhythms in the hamster brain, 25 hamsters were maintained in the previously described environmental conditions for 1 month. On the day of the experiment, the animals were sacrificed in groups of five at 0600, 1200, 1600, 2000, and 2400. In this study, only the brainstem was dissected. Norepinephrine in this brain area was extracted and quantitated by using the method of Shellenberger and Gordon (ref. 8-24).

In a third study, animals were sacrificed in groups of six at 0800, 1400, 1800, and 0100. The brainstem, the diencephalon, and the cerebral cortex of each of these animals were collected; norepinephrine was extracted by using the Maickel et al. method (ref. 8-19) and quantitated with the Chang method (ref. 8-22).

RESULTS

Graphs representative of the daily rhythmic changes observed in serotonin content in the brainstem and cerebral cortex of adult male AJAX mice are shown in figure 8-1. Only one peak in serotonin content is observed in the mouse cerebral cortex. As shown in figure 8-1, the peak (P) level of serotonin content in the cerebral cortex occurred at 1200 during the early light phase (with illumination occurring from 0900 to 2100), whereas the lowest or trough (T) level occurred late in the dark phase at 0400. Two peaks in serotonin content are typically seen over the 24-hour day in the brainstems of male mice. In figure 8-1, these peaks are observed at 0800 (just before the onset of the light phase) and at 2400 (3 hours after the onset of the dark phase). In figure 8-1, troughs in the daily rhythm in brainstem serotonin occur at 1600 and 0400. The lowest level of brainstem serotonin is observed late in the dark phase at 0400.

The clock hours of the peak and trough serotonin levels found in the cerebral cortex of adult male mice in three separate experiments are indicated in table 8-I. Balb/C mice were used in the experiment conducted April 26, 1972, while AJAX mice were studied in the experiments conducted May 30, 1972, and June 20, 1972. In the experiment performed May 26, the peak in serotonin content was observed at 0400, late in the dark phase, but was again equally as high at 0800. It could not be determined whether serotonin levels remained high throughout the 4-hour period between 0400 and 0800. In that same experiment, the trough in the serotonin rhythm in the cerebral cortex was observed at 2400, also during the dark phase. In the experiment performed May 30, the serotonin peak was observed at 1200, during the early light phase; and the trough was observed at 0400, late in the dark phase. In the experiment performed June 20, the peak in serotonin content in the cerebral cortex was again observed at 1200 and the trough was observed again in the dark phase at 2400. In all three experiments, the lowest level of serotonin was observed during the dark phase of the lighting regime.

The clock hours of the first and second peaks (P1 and P2) in the serotonin rhythm in the brainstem of adult male mice for five separate experiments are shown in table 8-II. The clock hours of the first and second troughs (T1 and T2) are also shown for these experiments. Balb/C mice were used in the first three experiments, which were performed on February 15, 1972, February 23, 1972,

and April 26, 1972; and AJAX mice were studied in the May 30, 1972, and June 20, 1972, experiments. In the experiment conducted February 15, 1972, peaks in the serotonin content were observed at 0800 and at 2000, while the troughs were observed at 1600 and 0400. In the experiment performed February 23, 1972, peaks in the serotonin content were again observed at 0800 and 2000, and the troughs occurred at 1600 and 0200. In the April 26 study, the peaks occurred at 1200 and 0400, and the troughs occurred at 0800 and 2400. The clock hours of the peaks in brainstem serotonin content were identical (0800 and 2400) for the experiments of both May 30 and June 20. The clock hours of the troughs in the former experiment occurred at 1600 and 0400 and those in the latter occurred at 1200 and 0400. In all the experiments except that of February 23, the lowest mean level of serotonin was observed during the dark phase of the lighting regime.

Although they were sought in each experiment, significant daily changes in the content of norepinephrine have not been consistently found in any brain area in either Balb/C or in AJAX mice. It is thus reasonable to assume that daily changes in norepinephrine content are not characteristic of the mouse brain.

Changes in the content of 5-HIAA, the primary metabolite of serotonin, in the cerebral cortex of mice with changes in the time of day are shown in figure 8-2. The results of three different experiments are compared in this figure. The changes in the content of 5-HIAA in the cerebral cortex with time of day were statistically significant ($P < 0.05$) in all three experiments. In experiment 1, the peak in 5-HIAA content was observed at 2000 with equally low levels observed at 0800 and 2400. In experiment 2, two peaks in 5-HIAA seem to be present. Both occurred during the dark phase, one at 0800 and the second at 2400. Two almost equally low levels in the 5-HIAA content were observed in experiment 2, one at 1600 and the second at 0400. In experiment 3, one peak in the 5-HIAA content was clearly evident. This peak occurred at 2400, whereas the lowest level of 5-HIAA occurred at 1600.

Changes in the levels of 5-HIAA in the brainstem with time of day were not always statistically significant. The time periods when brainstem 5-HIAA were high or low also varied from one experiment to another. Further studies on daily changes in brainstem 5-HIAA are presently underway, but no data on 5-HIAA content in the brainstem will be presented here.

Figure 8-3 summarizes the results of a preliminary experiment in which the turnover of serotonin in the cerebral cortex of AJAX mice was determined during three separate times over a 24-hour day. The turnover of serotonin was determined by measuring the accumulation of 5-HIAA in the cerebral cortex after the active transport of this metabolite from the brain was inhibited by the injection of probenecid (200 mg/kg) (ref. 8-25). At 1000, the 5-HIAA content had increased 75 percent over that observed in mice sacrificed 2 hours earlier at the time when the experiment mice received their probenecid injection. At 1800, the probenecid-injected animals had accumulated 45 percent more 5-HIAA than the controls. At 0400, the 5-HIAA content of the probenecid-injected animals was only 5 percent greater than that observed in the control animals sacrificed 2 hours earlier. The decrease in the total level of 5-HIAA accumulated in the cerebral cortex of the probenecid-treated animals at 0400 was statistically significant ($P < 0.01$) compared to the levels found in the probenecid animals

at 1000 and 1800. The content of 5-HIAA in the cerebral cortex of the control animals sacrificed at 0200 was greater than that observed in the control animals at 0800 and 1600, but this increase in 5-HIAA content in the control animals was not statistically significant.

Figures 8-4 to 8-6 summarize the results of an experiment to test the effect of a restricted feeding regime on the daily serotonin rhythm observed in the whole brain of AJAX mice. As described in the section entitled "Methods," the control mice had food available ad libitum, whereas the experimental animals were given food for only 4 hours, between 1000 and 1400 daily. Preliminary studies indicated that these mice normally eat almost 80 percent of their food during the dark phase of a 12:12 LD regime.

In figure 8-4, the daily changes in whole plasma tryptophan are compared for the control and the experimental animals. The changes in plasma tryptophan with time of day in both groups of animals were highly significant ($P < 0.01$). Figure 8-4 clearly shows that the rhythm in plasma tryptophan was markedly altered in the experimental animals maintained on the daily feeding regime. The highest levels of whole plasma tryptophan in the control animals occurred during the dark phase at 2400. This is approximately the same period in which the control mice were eating the major portion of their daily food intake. In the experimental animals, the highest level of plasma tryptophan occurred during the light phase at 1200. This time period falls at the midpoint of the time interval when food was available to these experimental animals.

In figure 8-5, the diurnal changes in brain tryptophan content are compared for the control mice in relation to those on the feeding regime. The differences in brain tryptophan with time of day were statistically significant for both the control and the experimental animals ($P < 0.01$). Unlike the situation with regard to whole plasma tryptophan, the phasing of the daily rhythm in brain tryptophan was not appreciably affected by the feeding regime. The highest levels of brain tryptophan in both groups of animals occurred at 0800 (1 hour before the onset of the light phase of the lighting regime). The levels of tryptophan in the brains of the control animals and the experimental animals were essentially the same at corresponding time points throughout the 24-hour period. Only at 2000 do the standard errors of the means for the control and experimental animals not overlap. Even at this time period, the mean levels of brain tryptophan in the two groups of animals were not statistically significant.

The daily rhythms in brain serotonin in the experimental and the control animals are compared in figure 8-6. The differences in serotonin content with time of day were statistically significant in both groups of animals ($P < 0.005$). As with brain tryptophan, there was no apparent difference in the phasing of the daily serotonin rhythms in the experimental compared to the control animals. However, the content of serotonin in the brains of the control animals was significantly greater than that of the experimental animals at 0800 and 1200 ($P < 0.025$). When the results of figures 8-5 and 8-6 are compared, the changes in brain serotonin with time of day seem to parallel similar changes in the content of brain tryptophan.

Other experiments were concerned with determining the nature of diurnal changes in norepinephrine content in the brainstem, the diencephalon, and the cerebral cortex of male golden hamsters. The results of one such experiment are summarized in figure 8-7. In this study, statistically significant changes in the content of norepinephrine were observed in the brainstem, the diencephalon, and the cerebral cortex. The highest levels of norepinephrine in each of these three brain areas were observed at the end of the light phase (2000). The highest levels of norepinephrine in the brainstem, the diencephalon, and the cerebral cortex represented respective increases of 24 percent, 46 percent, and 36 percent over the lowest levels of norepinephrine observed in each area.

In the first experiment on hamsters, norepinephrine had been extracted from each of the brain areas by the method of Maickel et al. (ref. 8-19). In the second experiment on hamsters, only the brainstem was studied, and the norepinephrine in this brain area was extracted by using the Shellenberger and Gordon procedure (ref. 8-24). The results were very similar to those found for the brainstem in the first study (fig. 8-8). The peak of norepinephrine content was observed at 1600 and the lowest content of norepinephrine was observed at 0600, the end of the dark phase. The lower levels of norepinephrine observed in the first experiment suggest that the Maickel and the Shellenberger methods were giving different values for norepinephrine content in the hamster brain. However, in the third experiment shown in figure 8-8, the levels of norepinephrine found in the hamster brainstem by the Maickel method were in close agreement with those found in the second experiment by the Shellenberger method. In this third study, the peak level of norepinephrine in the hamster brainstem was observed at 1800, and the norepinephrine was almost equally low at 0800 and 1400.

DISCUSSION

As shown in figure 8-1 and table 8-I, significant changes in the content of serotonin are observed in the cerebral cortex of adult male mice. In AJAX mice, only one peak in serotonin content is observed, usually early in the light phase at 1200. Visual observations indicate that this is the period in which these animals are relatively inactive or asleep. The lowest level of serotonin in the cerebral cortex is observed in the dark phase at 2400 and 0400. This is the period in which the animals are active and are eating approximately 80 percent of their normal daily food consumption. As shown in table 8-I, Balb/C mice also have a similar daily rhythm in cerebral cortex serotonin. The peak in serotonin content in this strain occurred somewhat earlier at 0400 and 0800. However, statistically significant changes in serotonin content with time of day were not always observed in the Balb/C strain of mice.

Statistically significant changes in serotonin content with time of day have been observed in the brainstem of mice in every experiment to date. Characteristically, there are two peaks in serotonin content in the brainstem (fig. 8-1). The phasing (clock hour of peaks and troughs) of the serotonin rhythm in the brainstem is almost the same from experiment to experiment whether Balb/C or AJAX mice are studied (table 8-II). The first peak in the

serotonin content in the brainstem is observed usually near the end of the dark phase (0800) or during the early light phase (1200) (table 8-II). The first trough is observed in the light phase, usually at 1600 or at 1200. In one study, the first trough was observed at 0800. The second peak is observed during the dark phase (2400 or 0400) or just before the onset of the dark phase at 2000 (table 8-II). The second trough is always found during the dark phase (2400 or 0400) and in almost all cases represents the lowest level of serotonin observed during the 24-hour period.

When changes in 5-HIAA (the major metabolite of serotonin) content are measured at different time periods over the 24-hour day, one peak in the content of this substance is usually seen (fig. 8-2). This peak occurs during the dark phase (2400) or just before the onset of the dark phase (2000). The second peak (observed in experiment 2 at 0800) may represent the same peak observed at 2400 during the subsequent dark phase. Throughout the 24-hour period, the 5-HIAA levels in the cerebral cortex are usually inversely correlated with the levels of serotonin. If changes in the 5-HIAA content in the cerebral cortex are correlated with changes in serotonin metabolism, the inverse relationship between 5-HIAA and serotonin content may imply that the daily serotonin rhythm is due to changes in the rate of its metabolism. If this were true, then serotonin metabolism would seem to be greater during the dark phase. On the other hand, the changes in 5-HIAA content over the 24-hour period may not reflect changes in serotonin metabolism but rather may reflect changes in the rate at which 5-HIAA is actively transported from the brain.

When the turnover of serotonin was determined by measuring the accumulation of 5-HIAA after probenecid injection, a significant decrease in serotonin turnover was observed in the dark phase (0400) compared to the light phase (1000 and 1800). This supports the recent report of Hery et al. (ref. 8-26) who found a decrease in serotonin synthesis in the rat brain during the dark phase. These results support the suggestion of Friedman and Walker (refs. 8-3 and 8-4) that the high levels of serotonin observed in rat brain during the light phase may be functionally related to the decreased activity of these animals during the light phase. The results of this experiment on serotonin turnover suggest that the daily changes in 5-HIAA content observed in the previous experiments are a result of changes in the transport of this metabolite out of the brain rather than a result of changes in the turnover of serotonin. At this writing, only one experiment measuring the turnover of serotonin has been performed. Other studies are needed before any conclusion about the relationship of serotonin turnover to the daily serotonin rhythm can be determined.

Fernstrom and Wurtman (ref. 8-27) have suggested that the daily rhythm in rat brain serotonin may be the result of similar daily variations in plasma tryptophan. Perez-Cruet et al. (ref. 8-28) have proposed that the diurnal changes in brain serotonin may be secondary to diurnal cycles in feeding and to the influence of food intake on circulating levels of plasma tryptophan. The results of the experiment summarized in figure 8-6 do not seem to support a major role for daily food intake as a factor involved in the regulation of the daily serotonin rhythm in mouse whole brain. As shown in figure 8-6, the phasing of the daily serotonin rhythms in the whole brains of control and experimental mice was the same even though the experimental mice were given food for only a 4-hour period, from 1000 to 1400 daily. The control mice

normally eat 80 percent of their daily food consumption during the dark phase (2100 to 0900) of a 12:12 LD regime. The content of brain serotonin in the experimental mice was significantly lower than that of the controls at 0800 and 1200 ($P < 0.025$). These time periods represent times just before and during the period of food consumption for the experimental animals. It is likely that the serotonin contents in the brains of the experimental animals are lower at these time points because these animals are awake and eating at this time instead of resting, as the control animals are. The content of brain serotonin in the experiment mice at 0800 is still the highest observed during the 24-hour period.

Food intake does not seem to be a major factor affecting the daily rhythm of brain tryptophan in mice. The results outlined in figure 8-5 show that the restricted feeding regime did not alter the brain tryptophan content or the daily rhythm in the levels of tryptophan in the whole brain of the experimental mice compared to controls. A comparison of the serotonin rhythms (fig. 8-6) and the brain tryptophan rhythms (fig. 8-5) for both the control and the experimental animals does indicate a close correlation between the daily rhythms in brain serotonin and tryptophan. The correlation may be only coincidental because the proportion of total brain tryptophan present in serotonergic neurons, and thus available for serotonin synthesis, must be relatively small.

Figure 8-4 indicates that food intake may have an important role in regulating the daily rhythm in total plasma tryptophan. In the control animals, the peak in plasma tryptophan content occurred during the dark phase (2400), which corresponds to the time that these animals were consuming approximately 80 percent of their daily food intake. When the experimental mice were forced to eat at another time during the day (between 1000 and 1400 of the light phase), the plasma tryptophan rhythm was altered so that the peak was again observed during the time of food consumption (1200). Similar changes in the daily rhythm in plasma tyrosine were observed in a similar study in which rats were fed only during a daily 4-hour period (ref. 8-29).

Although the rhythm in whole plasma tryptophan was altered by the feeding regime, the phasing of the brain serotonin rhythm was not. These results also argue against a role for whole plasma tryptophan as a major factor involved in the regulation of the daily rhythmic changes in brain serotonin. No positive correlation was observed between plasma tryptophan and brain serotonin even in the control animals. The peak of whole plasma tryptophan in the control mice occurred at 2400, almost 8 hours before the peak in brain serotonin. The present results cannot completely rule out a role for plasma tryptophan in producing the brain serotonin rhythm, because only total plasma tryptophan was measured. A portion of plasma tryptophan is bound to albumin and thus is not immediately available for uptake by the brain or other tissues (ref. 8-30). Studies to compare daily changes in free plasma tryptophan with daily changes in brain serotonin are anticipated.

The present results also provide no evidence for a major role of total plasma tryptophan in the regulation of the content of brain tryptophan or of the daily rhythm in the content of brain tryptophan. Perez-Cruet et al. (ref. 8-28) also concluded that plasma and brain tryptophan were regulated by different mechanisms.

The rhythmic changes with time of day in the norepinephrine content in hamster brain correspond closely in the brainstem, the diencephalon, and the cerebral cortex (fig. 8-7). The clock hours of peaks and troughs of the norepinephrine content in these brain areas also show close agreement when separate experiments are compared (fig. 8-8). In general, the highest level of norepinephrine is observed late in or at the end of the light phase, and the lowest level normally occurs during the middle of or end of the dark phase. In the third experiment (fig. 8-8), the lowest level of norepinephrine was observed during the early light phase, but it seems very likely from the data that norepinephrine content would have been equally low during the late dark phase had a sacrifice been performed at that time. Indeed, a subsequent experiment (not discussed in this report) has confirmed this conclusion. The demonstration of similar rhythms in norepinephrine in the hamster brainstem, whether the Shellenberger or the Maickel method was used for the extraction of norepinephrine, strongly supports the conclusion that the rhythm observed is indeed a true rhythm in norepinephrine. A similar daily norepinephrine rhythm was reported for whole hamster brains by Lew (ref. 8-13).

SUMMARY

1. Reproducible rhythmic changes in serotonin content have been observed in the brainstem and cerebral cortex of AJAX mice.

2. The daily serotonin rhythm in the mouse cerebral cortex has one peak, which normally occurs early in the light phase.

3. The daily serotonin rhythm in the mouse brainstem is usually characterized by two peaks: one in the light phase and one that usually appears in the dark phase. The lowest level of serotonin observed in the brainstem over the 24-hour period usually occurs later in dark phase.

4. Reproducible changes in 5-HIAA content with time of day are also observed in the cerebral cortex of mice. These daily changes in 5-HIAA content may be more the result of a change in the rate of transport of this serotonin metabolite out of the brain than to a change in the turnover of serotonin.

5. Preliminary results indicate that serotonin turnover in the cerebral cortex of the mouse is greater during the light phase than during the dark phase of a lighting regime that consists of 12 hours of light and 12 hours of darkness.

6. The daily serotonin rhythm in the whole mouse brain is correlated with a similar rhythm in brain tryptophan. However, the daily rhythm in neither brain serotonin nor brain tryptophan seems to be dependent upon or secondary to daily cyclic variations in food consumption or to whole plasma tryptophan.

7. The rhythmic daily changes in whole plasma tryptophan in mice do seem to be related to if not dependent upon daily variations in the food consumption.

8. Reproducible changes in norepinephrine content were observed in the brainstem, diencephalon, and cerebral cortex of adult male golden hamsters.

REFERENCES

8-1. Albrecht, P.; Visscher, M. B.; Bittner, J. J.; and Halberg, F.: Daily Changes in 5-hydroxytryptamine Concentration in Mouse Brain. Proc. Soc. Exp. Biol. Med., vol. 92, 1956, pp. 703-706.

8-2. Dixit, Balwant N.; and Buckley, Joseph P.: Circadian Changes in Brain 5-hydroxytryptamine and Plasma Corticosterone in the Rat. Life Sci., vol. 6, no. 7, Apr. 1967, pp. 755-758.

8-3. Friedman, Alexander H.; and Walker, Charles A.: Circadian Rhythms in Rat Mid-Brain and Caudate Nucleus Biogenic Amine Levels. J. Physiol., vol. 197, no. 1, July 1968, pp. 77-85.

8-4. Friedman, Alexander H.; and Walker, Charles A.: Rat Brain Amines, Blood Histamine and Glucose Levels in Relationship to Circadian Changes in Sleep Induced by Pentobarbitone Sodium. J. Physiol., vol. 202, no. 1, May 1969, pp. 133-146.

8-5. Scheving, L. E.; Harrison, W. H.; Gordon, P.; and Pauly, J. E.: Daily Fluctuation (Circadian and Ultradian) in Biogenic Amines of the Rat Brain. Am. J. Physiol., vol. 214, no. 1, Jan. 1968, pp. 166-173.

8-6. Quay, W. B.: Differences in Circadian Rhythms in 5-hydroxytryptamine According to Brain Region. Am. J. Physiol., vol. 215, no. 6, Dec. 1968, pp. 1448-1453.

8-7. Quay, W. B.: Regional and Circadian Differences in Cerebral Cortical Serotonin Concentrations. Life Sci., vol. 4, no. 3, Feb. 1965, pp. 379-384.

8-8. Okada, Fumihiko: The Maturation of the Circadian Rhythm of Brain Serotonin in the Rat. Life Sci., vol. 10, pt. I., no. 2, Jan. 1971, pp. 883-894.

8-9. Scapagnini, U.; Moberg, G. P.; Van Loon, G. R.; De Groot, J.; and Ganong, W. F.: Relation of Brain 5-hydroxytryptamine Content to the Diurnal Variation in Plasma Corticosterone in the Rat. Neuroendocrinology, vol. 7, 1971, pp. 90-96.

8-10. Asano, Yutaka: The Maturation of the Circadian Rhythm of Brain Norepinephrine and Serotonin in the Rat. Life Sci., vol. 10, pt. I, no. 15, Aug. 1971, pp. 883-894.

8-11. Reis, Donald J.; Weinbren, Marlene; and Corvelli, Anthony: A Circadian Rhythm of Norepinephrine Regionally in Cat Brain: Its Relationship to Environmental Lighting and to Regional Diurnal Variations in Brain Serotonin. J. Pharm. Exp. Therap., vol. 164, no. 1, Nov. 1968, pp. 135-145.

8-12. Morgan, W. W.; McFadin, L. S.; and Harvey, C. Y.: A Daily Rhythm in Norepinephrine Content in Regions of the Hamster Brain. J. Comp. Pharmac., vol. 4, 1973, pp. 47-52.

8-13. Lew, G. W.: Circadian Rhythms in Hamster Adrenal, Heart, Spleen, and Brain Content of Catecholamines. Anat. Rec., vol. 172, 1972, p. 355.

8-14. Matussek, N.; and Patschke, U.: Beziehungen des Schlaf-und Wachrhythmus zum Noradrenalin-und Serotonin-gehalt im. Z entratnervensystem von Hamstern. Med. Exp., vol. 11, 1963, pp. 81-87.

8-15. Bobillier, P.; and Mouret, J. R.: The Alterations of the Diurnal Variations of Brain Tryptophan, Biogenic Amines and 5-hydroxyindoleacetic Acid in the Rat Under Limited Time Feeding. Int. J. Neuroscience, vol. 2, 1971, pp. 271-282.

8-16. Zigmond, Michael J.; and Wurtman, Richard J.: Daily Rhythm in the Accumulation of Brain Catecholamines Synthesized From Circulating H^3 Tyrosine. J. Pharm. Exp. Therap., vol. 172, no. 2, Apr. 1970, pp. 416-422.

8-17. Hanin, I.; Massarelli, R.; and Costa, E.: Acetylcholine Concentrations in Rat Brain: Diurnal Oscillation. Science, vol. 170, no. 3955, Oct. 16, 1970, pp. 341-342.

8-18. Schreiber, Robert A.; and Schlesinger, Kurt: Circadian Rhythms and Seizure Susceptibility: Relation to 5-hydroxytryptamine and Norepinephrine in Brain. Physiol. Behav., vol. 6, no. 6, June 1971, pp. 635-640.

8-19. Maickel, Roger P.; Cox, Raymond H., Jr.; Saillant, Jean; and Miller, Francis P.: A Method for the Determination of Serotonin and Norepinephrine in Discrete Areas of Rat Brain. Int. J. Neuropharmac., vol. 7, no. 3, May-June 1968, pp. 275-281.

8-20. Curzon, G.; and Green, A. R.: Rapid Method for the Determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic Acid in Small Regions of Rat Brain. Brit. J. Pharma., vol. 39, July 1970, pp. 653-655.

8-21. Alpers, H. S.; and Himwich, H. E.: The Effects of Chronic Imipramine Administration on Rat Brain Levels of Serotonin, 5-hydroxyindoleacetic Acid, Norepinephrine, and Dopamine. J. Pharm. Exp. Therap., vol. 180, Mar. 1972, pp. 531-538.

8-22. Chang, C. C.: A Sensitive Method for Spectrophotofluorometric Assay of Catecholamines. Neuropharmacology, vol. 3, 1964, pp. 643-649.

8-23. Denckla, W. D.; and Dewey, Henry K.: The Determination of Tryptophan in Plasma, Liver, and Urine. J. Lab. Clin. Med., vol. 69, no. 1, Jan. 1967, pp. 160-169.

8-24. Shellenberger, M. Kent; and Gordon, J. H.: A Rapid, Simplified Procedure for Simultaneous Assay of Norepinephrine, Dopamine, and 5-hydroxytryptamine from Discrete Brain Areas. Anal. Biochem., vol. 3, no. 2, Feb. 1971, pp. 356-372.

8-25. Neff, Norton H.; and Tozer, T. N.: In Vivo Measurement of Brain Serotonin Turnover. Advanc. Pharma., vol. 6, pt. A, 1968, pp. 97-109.

8-26. Hery, Francis; Rouer, E.; and Glowinski, J.: Daily Variations of Serotonin Metabolism in the Rat Brain. Brain Res., vol. 43, 1972, pp. 445-465.

8-27. Fernstrom, John D.; and Wurtman, R. J.: Brain Serotonin Content: Physiological Dependence on Plasma Tryptophan Levels. Science, vol. 173, no. 3992, July 9, 1971, pp. 149-152.

8-28. Perez-Cruet, J.; Tagliamonte, A.; Tagliamonte, P.; and Gessa, G. L.: Changes in Brain Serotonin Metabolism Associated with Fasting and Satiation. Life Sci., vol. 11, 1972, pp. 31-39.

8-29. Coburn, S. P.; Seidenberg, M.; and Fuller, R. W.: Daily Rhythm in Plasma Tyrosine and Phenylalanine. Proc. Soc. Exp. Biol. Med., vol. 129, no. 2, Nov. 1968, pp. 338-343.

8-30. McMenamy, Rapier H.; and Oncley, J. L.: The Specific Binding of L-tryptophan to Serum Albumin. J. Biol. Chem., vol. 233, no. 6, Dec. 1958, pp. 1436-1447.

TABLE 8-I.- CLOCK HOURS OF PEAK AND TROUGH LEVELS OF SEROTONIN IN THE CEREBRAL CORTEX OF ADULT MALE MICE

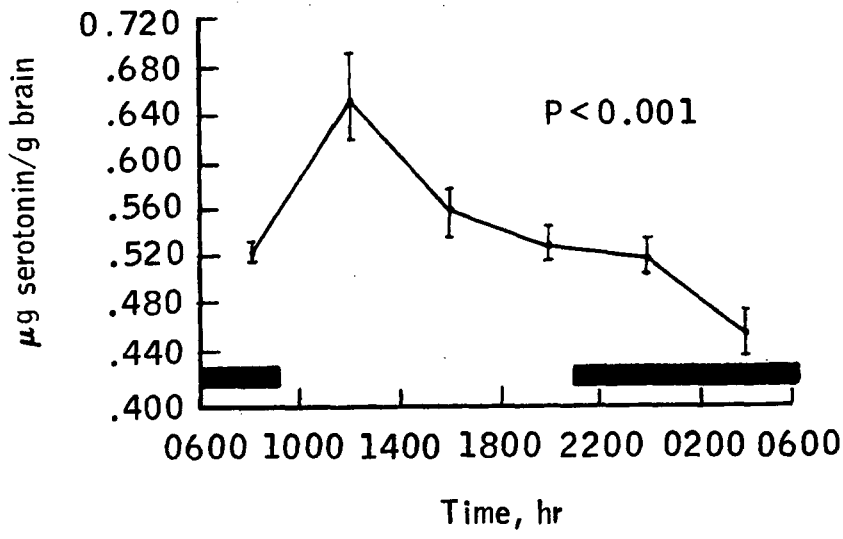
Date of experiment, 1972	Strain of mice	Time,[a] hr												Statistical significance
		0800	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	
Apr. 26	Balb/C	P								T		P		<0.01
May 30	AJAX			P								T		<.001
June 20	AJAX			P										<.01

[a]The horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

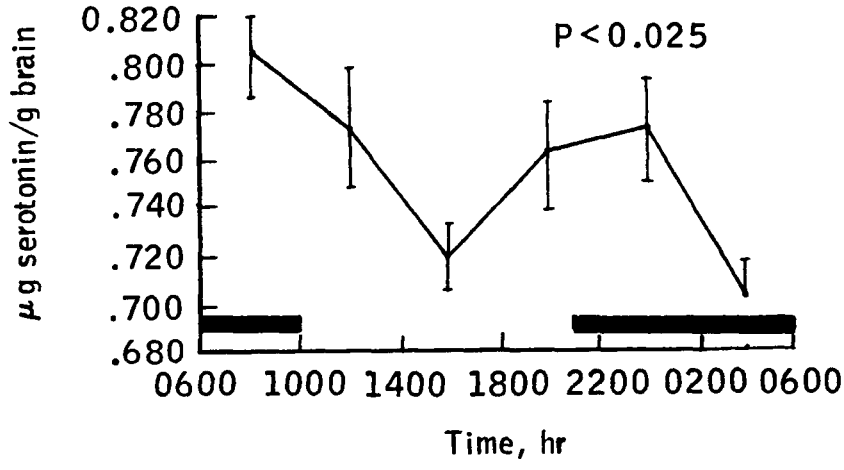
TABLE 8-II.- CLOCK HOURS OF PEAK AND TROUGH LEVELS OF SEROTONIN IN THE BRAINSTEM OF ADULT MALE MICE

Date of experiment, 1972	Strain of mice	Time,[a] hr												Statistical significance
		0800	1000	1200	1400	1600	1800	2000	2200	2400	0200	0400	0600	
Feb. 15	Balb/C	P1				T1		P2				T2		<0.05
Feb. 23	Balb/C	P1				T1		P2		T2				<.005
Apr. 26	Balb/C	T1		P1						T2		P2		<.001
May 30	AJAX	P1				T1				P2		T2		<.025
June 20	AJAX	P1		T1						P2		T2		<.01

[a]The horizontal black bar represents the dark phase of the 12:12 LD lighting regime.



(a) Cerebral cortex.



(b) Brainstem.

Figure 8-1.- Comparison of daily changes in serotonin content found in the cerebral cortex and brainstem of mice. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

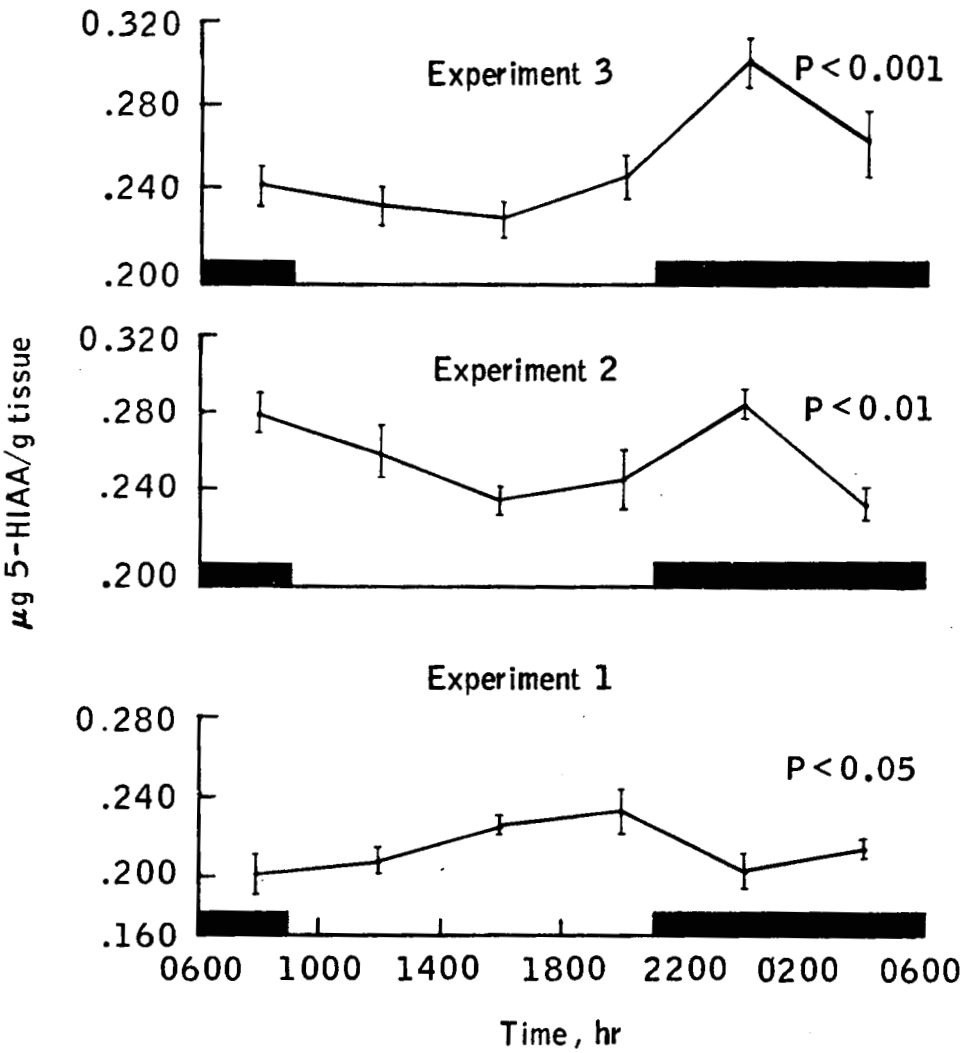


Figure 8-2.- Comparison of three separate studies showing daily changes in the content of 5-HIAA in the cerebral cortex of AJAX mice. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

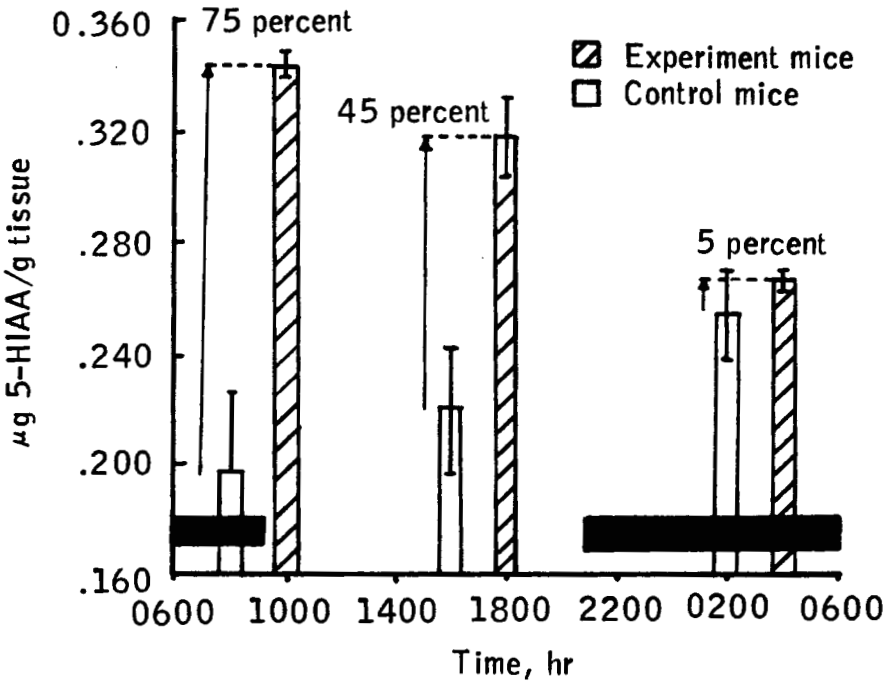


Figure 8-3.- Changes with time of day in probenecid-induced 5-HIAA accumulation in the cerebral cortex of mice. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

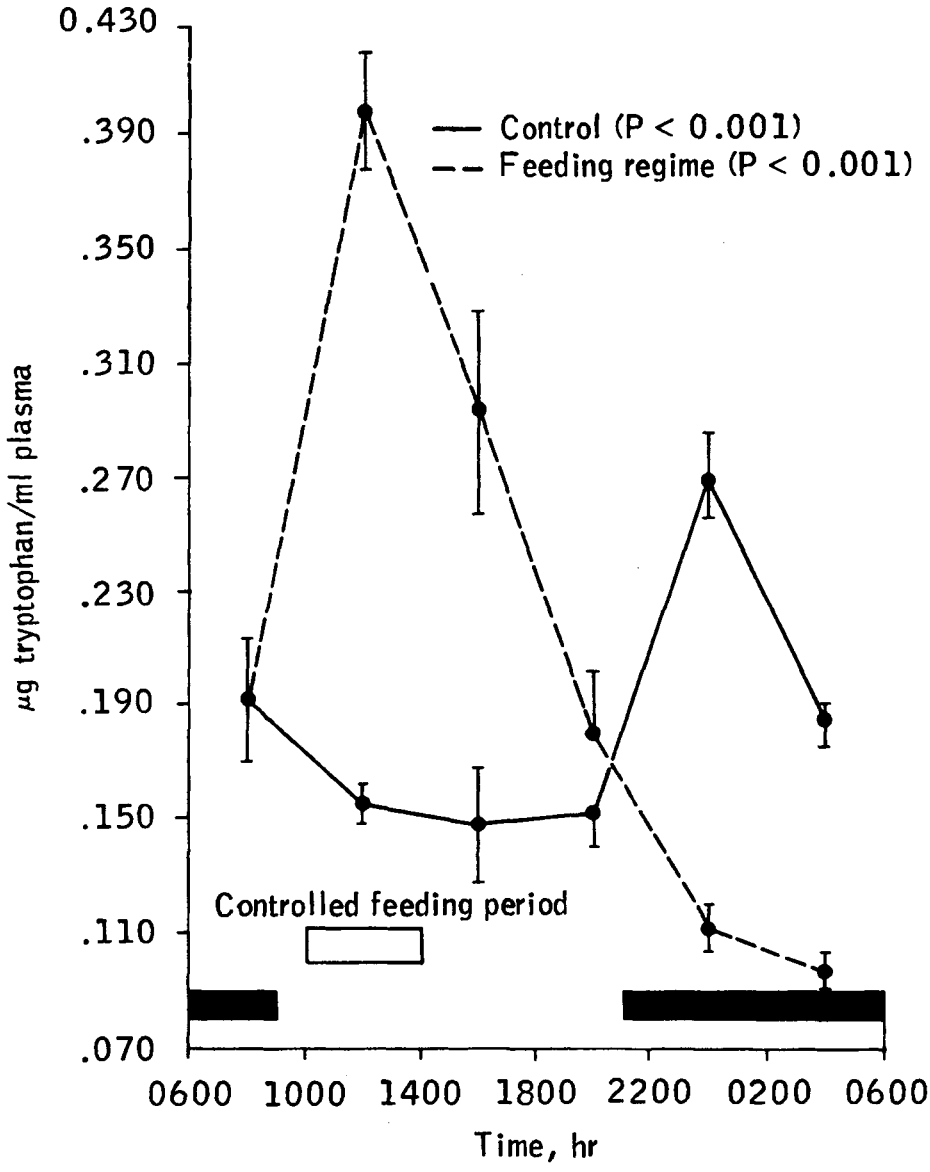


Figure 8-4.- Effect of a controlled feeding regime on the diurnal variation of plasma tryptophan content in the mouse. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

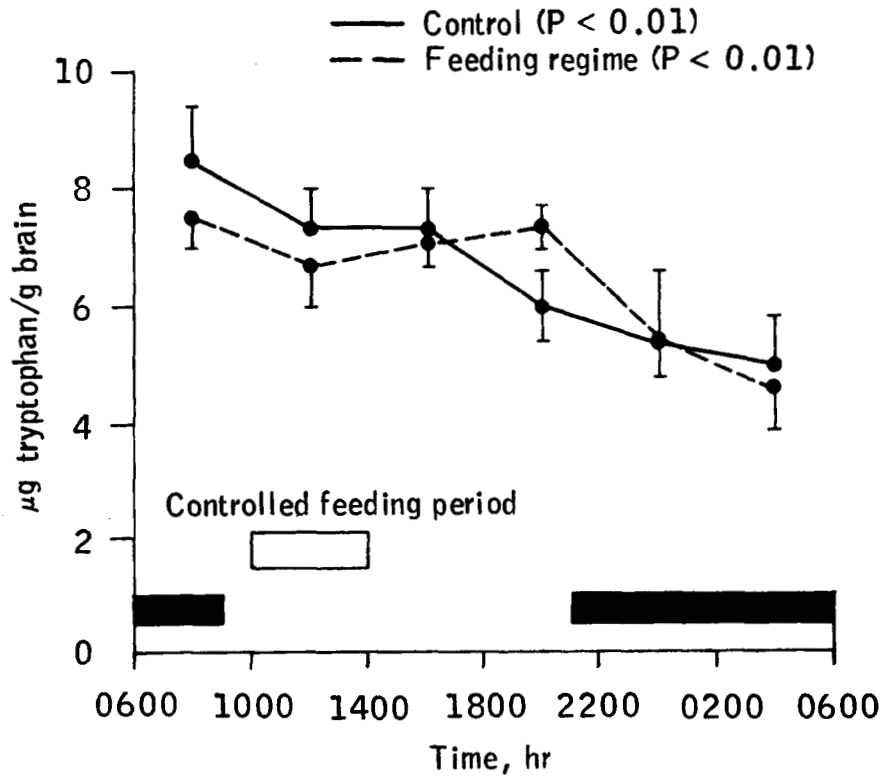


Figure 8-5.- Effect of a controlled feeding regime on the diurnal variation of tryptophan content in the mouse brain. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

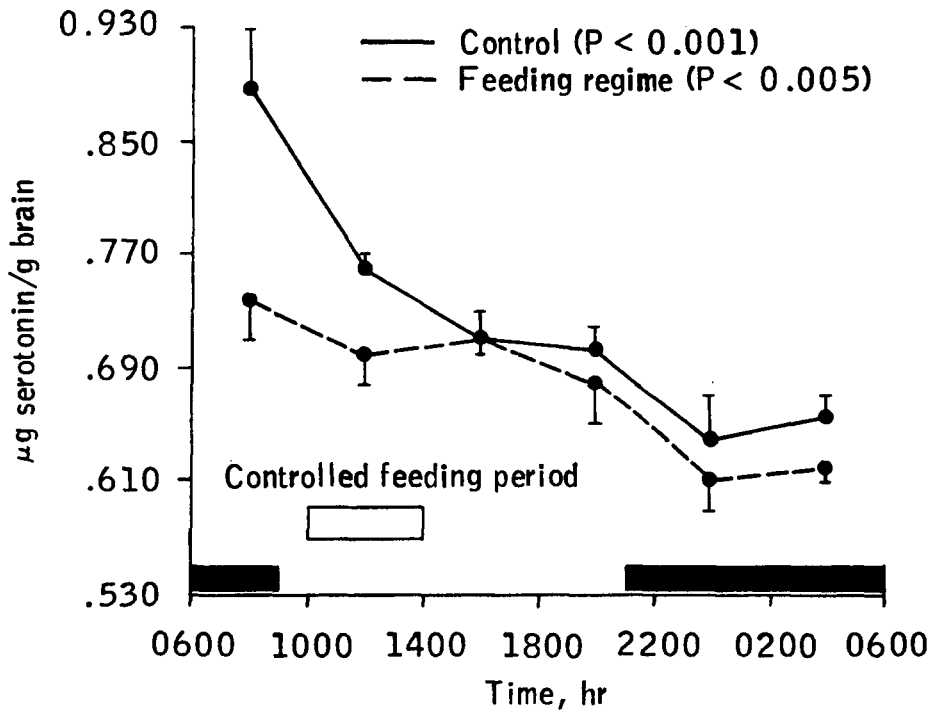
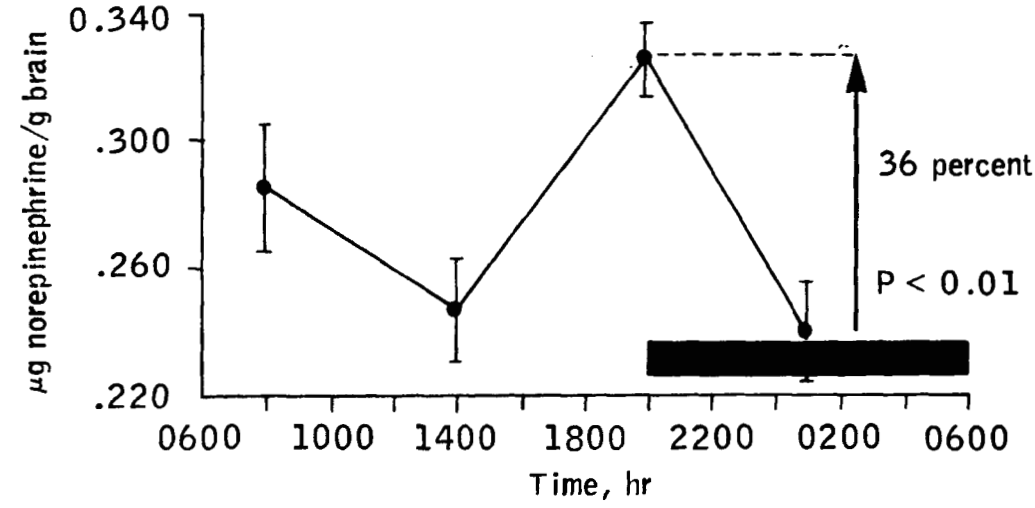
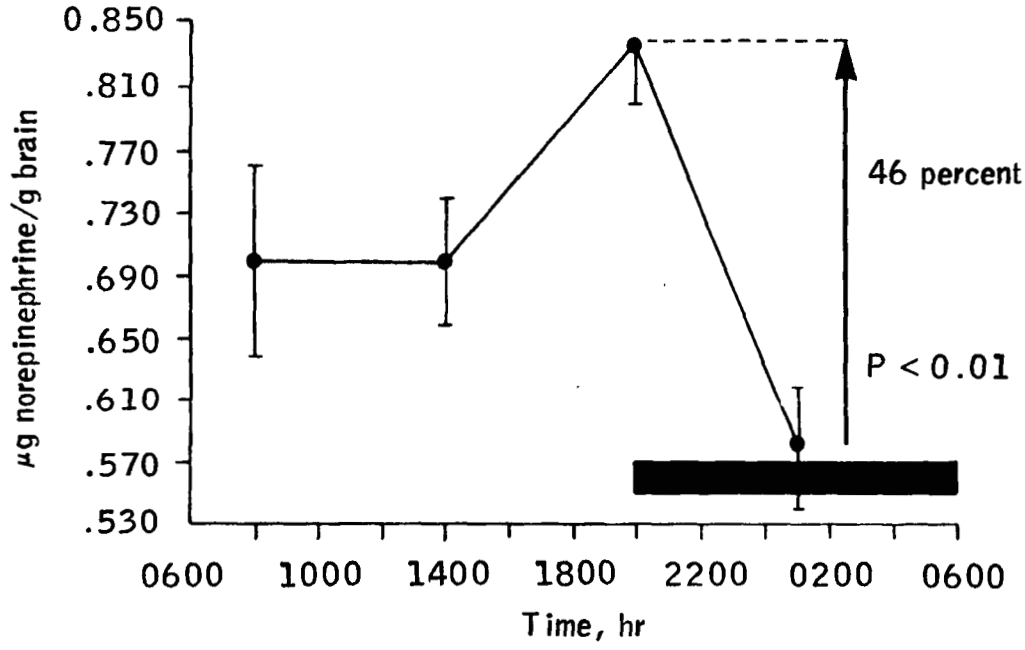


Figure 8-6.- Effect of a controlled feeding regime on the diurnal variation of serotonin content in the mouse brain. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 12:12 LD lighting regime.

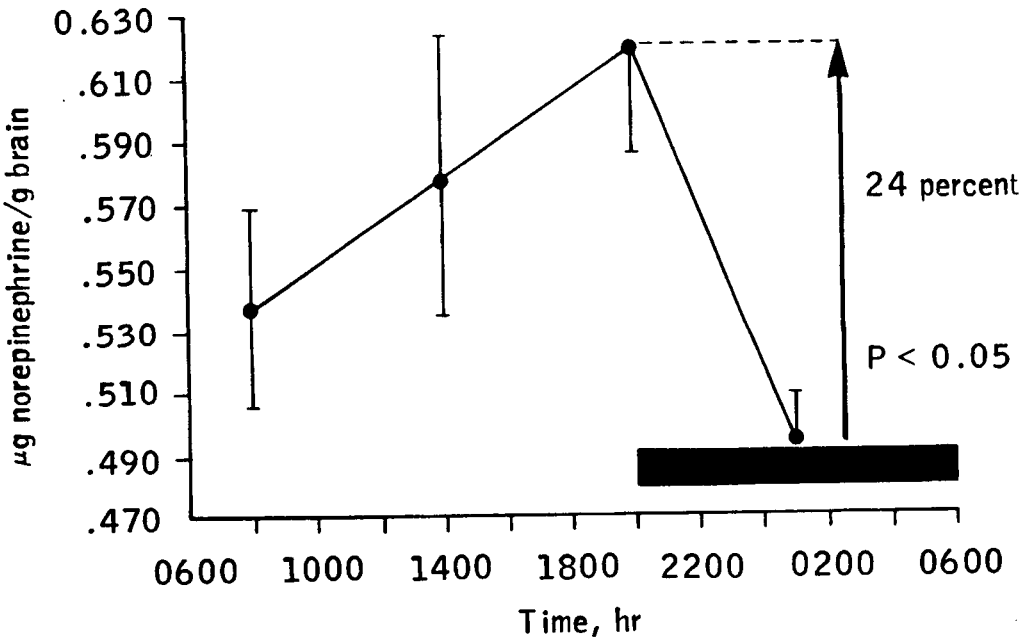


(a) Cerebral cortex.



(b) Diencephalon.

Figure 8-7.- Daily changes in norepinephrine content in areas of the hamster brain. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 14:10 LD lighting regime.



(c) Brainstem.

Figure 8-7.- Concluded.

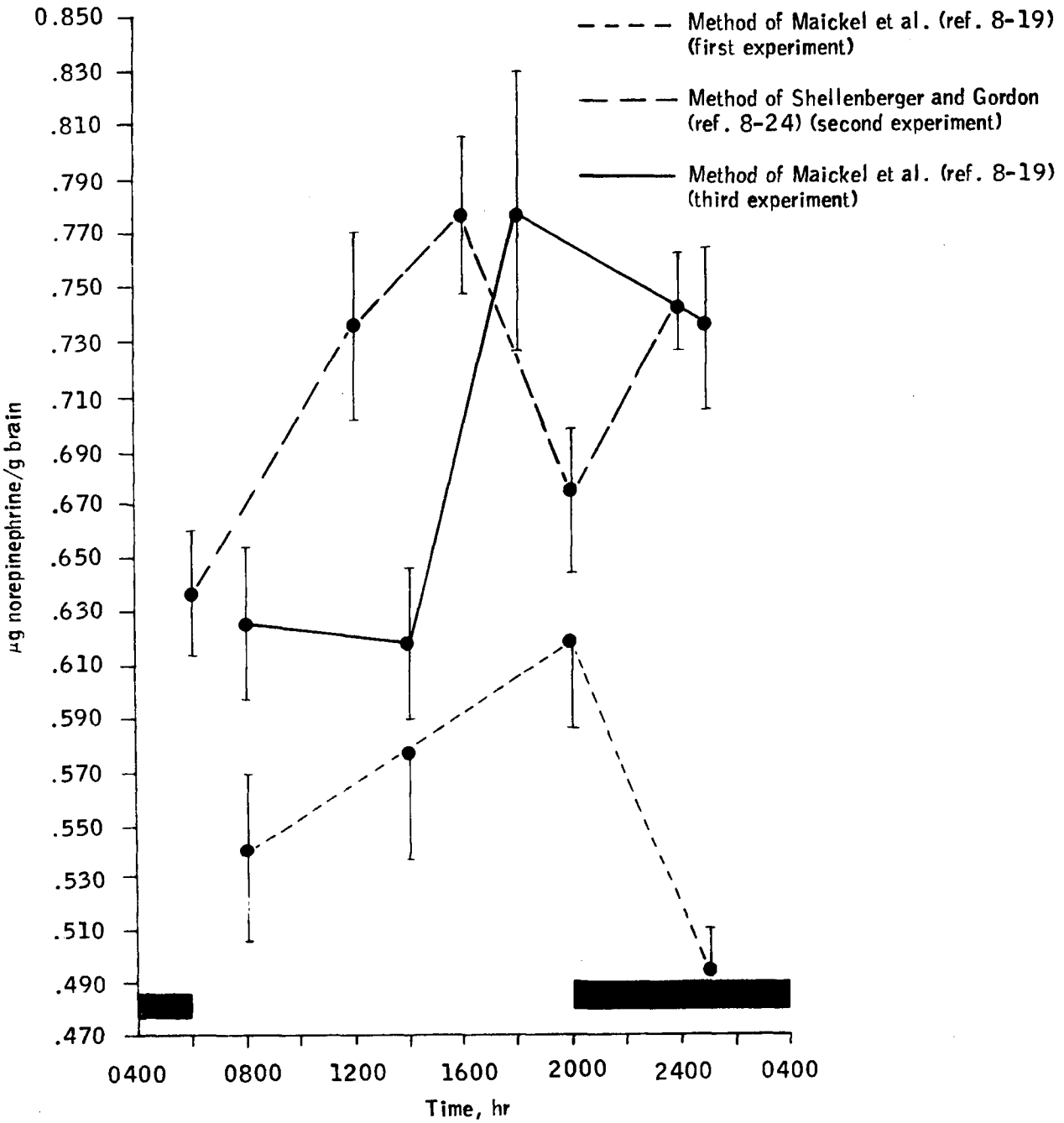


Figure 8-8.- Comparison of diurnal rhythms in norepinephrine content of hamster brainstem in separate experiments in which norepinephrine was determined by different methods. Brackets represent mean ± standard error; horizontal black bar represents the dark phase of the 14:10 LD lighting regime.

9. STUDIES OF SODIUM HOMEOSTASIS DURING SIMULATED WEIGHTLESSNESS: APPLICATION OF THE WATER IMMERSION MODEL TO MAN

By Murray Epstein, M.D.

INTRODUCTION

Reports of recent manned orbital flights have disclosed a significant natriuresis and weight loss following manned space flight (refs. 9-1 and 9-2). Attempts to investigate and define the volume depletion during weightlessness have been thwarted by the inherent difficulties in performing in-flight physiological investigations. A previous report from this laboratory proposed the use of the model of water immersion as an investigative tool for studying the circulatory mechanisms of plasma volume control and sodium homeostasis during weightlessness (ref. 9-3).

Previous studies carried out on sodium-depleted subjects demonstrated that water immersion to the level of the sternoclavicular notch (neck immersion) induced profound alterations of the renin-aldosterone system and renal sodium handling (refs. 9-3 and 9-4). Six hours of water immersion to the neck produced a 50 percent decrease in plasma renin activity (PRA) and urinary aldosterone excretion in normal subjects in balance on a 10-meq sodium diet. Although the rate of sodium excretion ($U_{Na}V$) during immersion was twentyfold greater than during the control period, the absolute increase in sodium excretion during water immersion was exceedingly small, possibly reflecting the limitations imposed by the sodium depleted and volume contracted state of the subjects. Therefore, an additional study was undertaken to assess the effects of water immersion on renal sodium management during a sodium intake (150 meq) more nearly approximating that of the normal diet (ref. 9-5). It was anticipated that such studies would further clarify the mechanisms involved in the natriuresis of water immersion.

DISCUSSION

Studies of renal sodium and potassium management were conducted on seven healthy male subjects who had consumed a diet containing 150 meq sodium and 100 meq potassium. Control and immersion studies were conducted on each subject on the third and fifth days of dietary equilibration, respectively. By that time, all subjects had achieved sodium balance. On study days, identical protocols were conducted as follows.

Each subject was given a 400-milliliter oral water load at 0800 hours. After voiding and completely emptying his bladder, the study began with the subject assuming a seated position for 6 hours. During the control studies, each subject sat quietly outside the immersion tank for the 6-hour period. During immersion, the seven subjects sat in the study tank immersed in water to the neck for the 6-hour period. Each subject voided spontaneously every hour. To maintain an adequate urine flow, 200 milliliters of water was administered orally every hour during each study. Sodium, potassium, and creatinine were measured in aliquots of the hourly urine collections. Blood was collected at 2-hour intervals throughout the study.

Immersion was in a waterproof tank described in reference 9-3. A constant water temperature of 307 ± 0.5 K (34 ± 0.5° C) was maintained by two heat exchangers with a combined output of 14 233 725 J/hr (13 500 Btu/hr) controlled by an adjustable temperature-calibrated control meter, the input to which was derived from two thermistors immersed at different water levels.

The effects of 6 hours of water immersion on sodium excretion are shown in figure 9-1. During control studies, the $U_{Na}V$ was constant, ranging from 58 to 71 μeq/min, despite a doubling of urine flow. In contrast, water immersion to the neck resulted in a highly significant increase in $U_{Na}V$ compared to the control period, beginning at hour 1 ($P < 0.005$). During the final 5 hours of study, $U_{Na}V$ during immersion was threefold to fourfold greater than during the comparable control periods ($P < 0.005$). Net sodium excretion during 6 hours of immersion was 71.6 ± 4.0 meq, threefold greater than 23.0 ± 2.5 meq during control ($P < 0.001$).

The present studies demonstrate that immersion to the neck for normal subjects in balance on a 150 meq sodium diet produces an earlier (hour 1 compared to hour 4) and more profound increase in the $U_{Na}V$ than in comparable subjects studied on a 10 meq sodium intake (fig. 9-1). The total quantity of sodium excreted during the 6 hours of immersion was almost tenfold greater than that found in patients studied on a 10 meq sodium diet (ref. 9-3).

The effect of water immersion on potassium excretion is shown in figure 9-2. The rate of potassium excretion (U_KV) did not vary significantly during the control studies, ranging from 51 to 68 μeq/min. Immersion produced a significant increase in U_KV beginning at hour 1 and persisting through hour 4. During the final 2 hours, U_KV was not significantly different from the control studies at a time that $U_{Na}V$ remained elevated. Net potassium excretion during immersion was 35.4 ± 2.8 meq, almost double the 20.9 ± 2.6 meq found during the control studies ($P < 0.005$).

The relationship between the increments in rate of urine flow (V) and $U_{Na}V$ during each period is depicted in figure 3. The mean percent increment in $U_{Na}V$ increased progressively during the first 3 hours of immersion, at a time that

the mean percent increment in V was decreasing. This demonstration of a dissociation between renal sodium and water management during immersion is consistent with recent evidence suggesting the presence of independent afferent mechanisms mediating antidiuretic hormone (ADH) and renin release. Brennan et al. (ref. 9-6) have demonstrated that increases in left atrial pressure result in decreases in ADH, without accompanying changes in PRA; on the other hand, increases in right atrial pressure result in significant decreases in PRA but no appreciable changes in plasma ADH.

Although the increase in $U_{Na}V$ observed during immersion is associated with a decrease in aldosterone excretion (refs. 9-3 and 9-4), the role of this decrease in mineralocorticoid activity in mediating the natriuresis remains unclear. Since effects on renal sodium management are manifest only after a 1- to 2-hour lag following changes in the aldosterone level (refs. 9-7 and 9-8), the early natriuresis observed here within the initial hour of immersion suggests that the natriuresis cannot be solely attributed to decreased aldosterone. This inference is further supported by the occurrence of a concomitant kaliuresis.

To assess the quantitative contribution of aldosterone suppression to the natriuresis of water immersion, an additional study was made, examining renal sodium management in normal subjects undergoing water immersion before and after administration of exogenous mineralocorticoid (ref. 9-9). Six healthy male subjects were studied following equilibration on a 150 meq sodium and 70 meq potassium diet. Following equilibration, each subject underwent a control study on day 3, followed on day 5 by a repeat study during deoxycorticosterone acetate (DOCA) administration (control + DOCA), on day 8 by water immersion to the neck (immersion), and on day 11 by immersion plus DOCA administration (immersion + DOCA). During immersion and immersion + DOCA, the subject sat in the tank immersed in water to the neck for 4 hours (0900 hours to 1300 hours), preceded and followed by 1 hour of quiet sitting outside the tank (preimmersion and recovery hours, respectively). Deoxycorticosterone acetate, 5 milligrams, was administered intramuscularly to each subject at 1300 and 1900 hours on the day preceding, and at 0100 and 0700 hours on the days of the control + DOCA and immersion + DOCA studies.

The effects of 4 hours of water immersion on sodium excretion are depicted in figure 9-4. Water immersion to the neck (immersion) resulted in a highly significant increase in $U_{Na}V$ as compared to the control, beginning with the initial hour of immersion ($P < 0.05$). During the final 3 hours of the study, $U_{Na}V$ during immersion was threefold to fourfold greater than during the comparable control periods. Although return to quiet sitting after 4 hours of immersion (recovery) was accompanied by a prompt decrease in sodium excretion as compared with the final hour of immersion ($P < 0.005$), $U_{Na}V$ was still elevated throughout the recovery hour as compared with the preimmersion hour ($P < 0.001$) and the comparable control period ($P < 0.01$) (fig. 9-5).

Pretreatment with DOCA for 24 hours (control + DOCA) resulted in a decrease in $U_{Na}V$ by more than 50 percent at each hour of study compared to the

control alone. Although DOCA pretreatment also blunted the natriuresis of immersion, $U_{Na}V$ was still threefold to fourfold greater during immersion + DOCA than during the comparable control + DOCA periods ($P < 0.01$). Despite a decrease in sodium excretion during the recovery hour compared to hour 4 of immersion + DOCA ($P < 0.05$), $U_{Na}V$ continued to exceed preimmersion values ($P < 0.05$) and the value during the comparable period of DOCA treatment alone ($P < 0.01$).

The present studies demonstrate that the administration of a potent mineralocorticoid in pharmacologic doses fails to abolish the natriuresis of water immersion. Although DOCA decreased $U_{Na}V$ during immersion by more than 50 percent, $U_{Na}V$ during immersion + DOCA again exceeded $U_{Na}V$ during control + DOCA by several fold.

The delayed rate of disappearance of the natriuresis observed during recovery following both immersion and immersion + DOCA further clarifies the mechanisms involved in immersion natriuresis. Both $U_{Na}V$ and the fractional excretion of sodium continued to exceed preimmersion values throughout the recovery hour (figure 9-5). The significance of this finding is underscored by the fact that it occurred in the face of marked volume contraction, as demonstrated by a significant negative sodium balance of 60.5 meq/4 hours, accompanied by a weight loss of 1.2 kilograms, as well as a significant antidiuresis occurring as early as the first 30 minutes of recovery. The persistence of the natriuresis in the face of progressive volume contraction suggests that the natriuretic stimulus was sufficiently potent to override the compensatory mechanisms that participate in the defense of volume homeostasis (ref. 9-1) and that a humoral factor rather than more rapidly acting hemodynamic and neural mechanisms may be involved in mediating the natriuresis. Such a humoral factor may be identical with the putative "natriuretic hormone" postulated by De Wardener over a decade ago (ref. 9-10).

Although the precise mechanisms and mediators remain to be clarified, the demonstrated ability of water immersion to reproducibly suppress renin and aldosterone and produce a significant natriuresis suggests that the model of water immersion may constitute a useful investigative tool for studying the circulatory mechanisms of plasma volume control and sodium homeostasis during weightlessness, and specific countermeasures for the management of the natriuresis of manned space flight.

REFERENCES

9-1. Gauer, O. H.; Henry, J. P.; and Behn, C.: The Regulation of Extracellular Fluid Volume. Ann. Rev. Physiol., vol. 32, 1970, pp. 547-595.

9-2. Lutwak, Leo; Whedon, G. Donald; LaChance, Paul A.; Reid, Jeanne M.; and Lipscomb, Harry S.: Mineral, Electrolyte, and Nitrogen Balance Studies of the Gemini-VII Fourteen-Day Orbital Space Flight. J. Clin. Endocrin., vol. 29, no. 9, Sept. 1969, pp. 1140-1156.

9-3. Epstein, Murray; and Saruta, Takao: Effect of Water Immersion on Renin-Aldosterone and Renal Sodium Handling in Normal Man. J. Appl. Physiol., vol. 31, no. 3, Sept. 1971, pp. 368-374.

9-4. Epstein, Murray; Fishman, L. M.; and Hale, H. B.: Dissociation of Aldosterone and 17-OHCS Release During Water Immersion in Normal Man. Proc. Soc. Exp. Biol. Med., vol. 138, 1971, pp. 939-942.

9-5. Epstein, Murray; Duncan, D. C.; Fishman, L. M.: Characterization of the Natriuresis Caused in Normal Man by Immersion in Water. Clin. Sci., vol. 43, Aug. 1972, pp. 275-287.

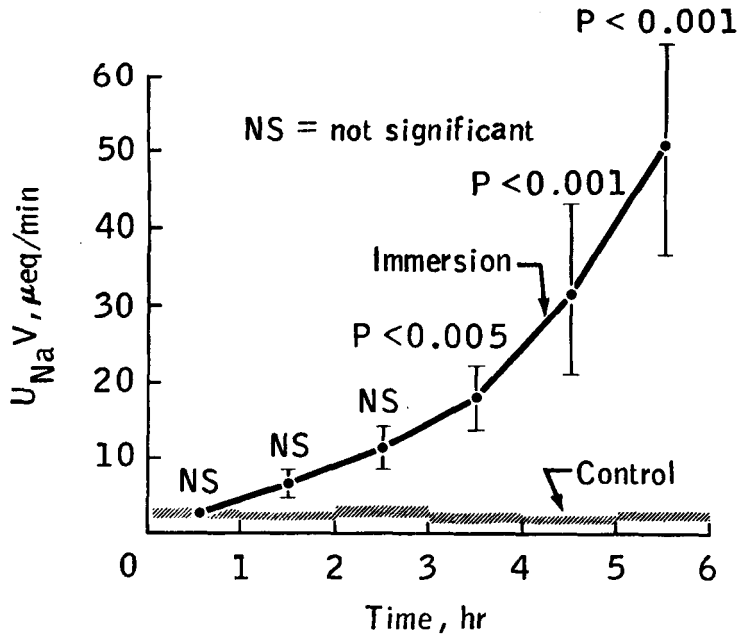
9-6. Brennan, Leonard A., Jr.; Malvin, Richard L.; Jochim, Kenneth E.; and Roberts, Donald E.: Influence of Right and Left Atrial Receptors on Plasma Concentrations of ADH and Renin. Am. J. Physiol., vol. 221, no. 1, July 1971, pp. 273-278.

9-7. Ross, E. J.; Reddy, W. J.; Rivera, A.; and Thorn, G. W.: Effects of Intravenous Infusions of dl-aldosterone Acetate on Sodium and Potassium Excretion in Man. J. Clin. Endocrin., vol. 19, 1959, pp. 289-296.

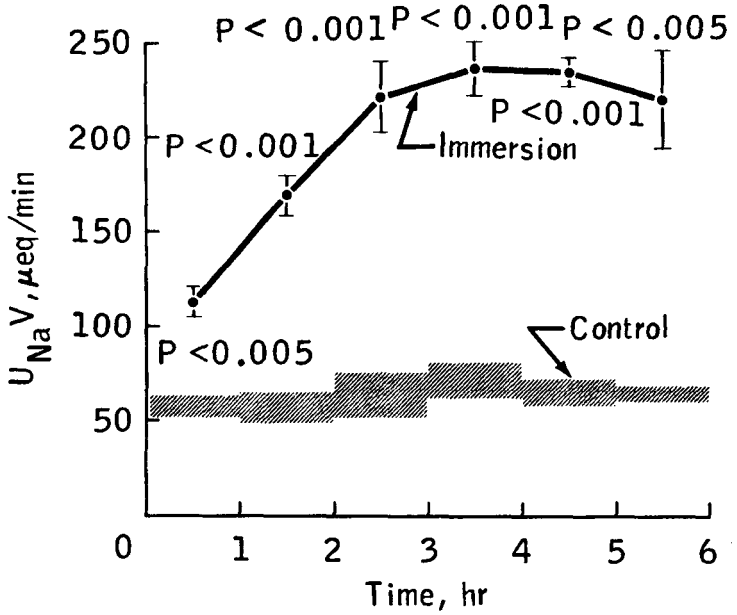
9-8. Crabbe, J.: Stimulation of Active Sodium Transport by the Isolated Toad Bladder with Aldosterone in vitro. J. Clin. Invest., vol. 40, Nov. 1961, pp. 2103-2110.

9-9. Epstein, Murray; Katsikas, James L.; and Duncan, David C.: The Role of Mineralocorticoids in the Natriuresis of Water Immersion in Man. Circ. Res., vol. 32, no. 2, Feb. 1973, pp. 228-236.

9-10. De Wardener, H. E.; Mills, I. H.; Clapham, W. F.; and Hayter, C. J.: Studies on the Efferent Mechanism of the Sodium Diuresis Which Follows the Administration of Intravenous Saline in the Dog. Clin. Sci., vol. 21, Oct. 1961, pp. 249-258.



(a) Low sodium diet, 10 meq sodium intake (N = 8).



(b) High sodium diet, 150 meq sodium intake (N = 7).

Figure 9-1.- Comparison of the effects of immersion on the rates of sodium excretion ($U_{Na}V$) for subjects in balance on low sodium and high sodium diets. Note the different scales on the ordinates; shaded areas represent the mean ± standard error for control studies; brackets represent mean ± standard error for immersion studies (from ref. 9-5).

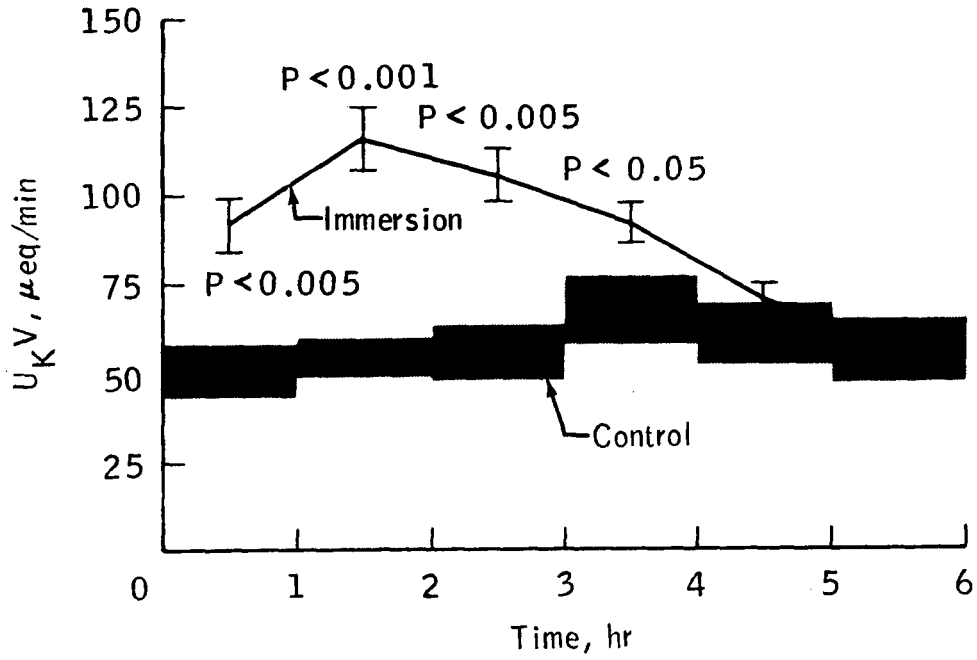


Figure 9-2.- Comparison of rates of potassium excretion during control and water immersion. Shaded area represents mean ± standard error for control studies; brackets represent mean ± standard error for immersion studies (N = 7).

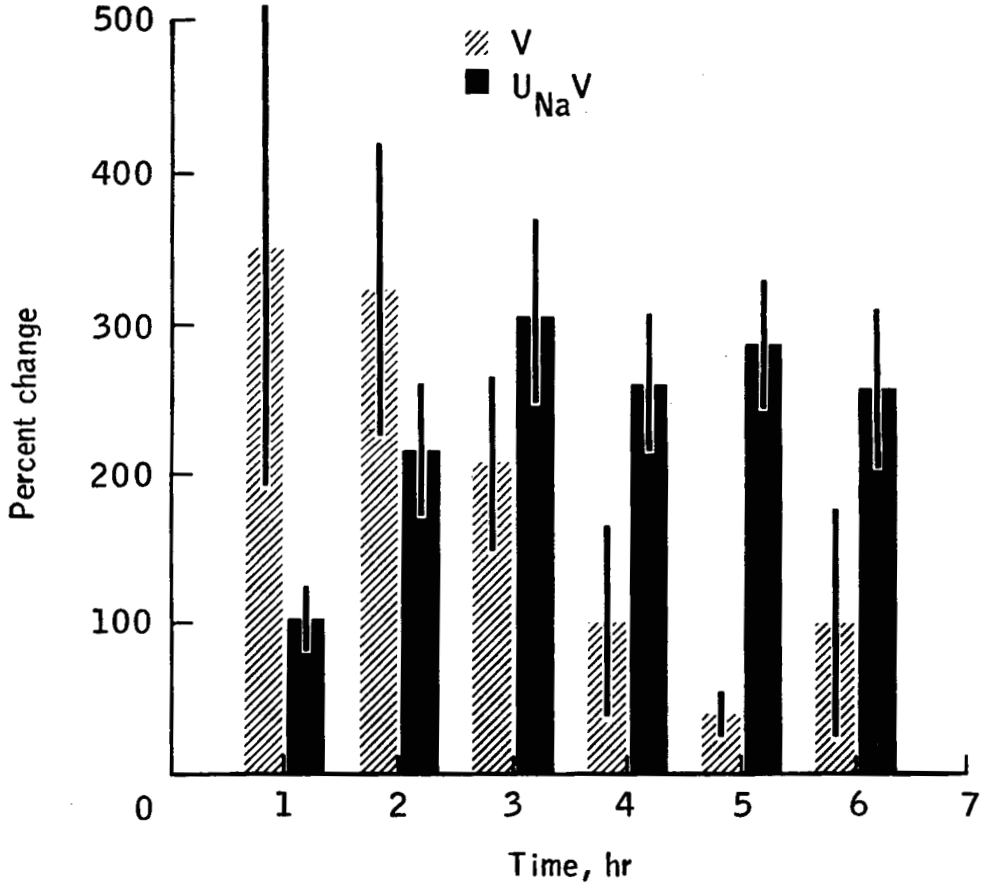


Figure 9-3.- Relationship of percent increments during immersion as compared to control, in rates of urine flow (V) and sodium excretion ($U_{Na}V$). Narrow vertical bars represent mean ± standard error (N = 7).

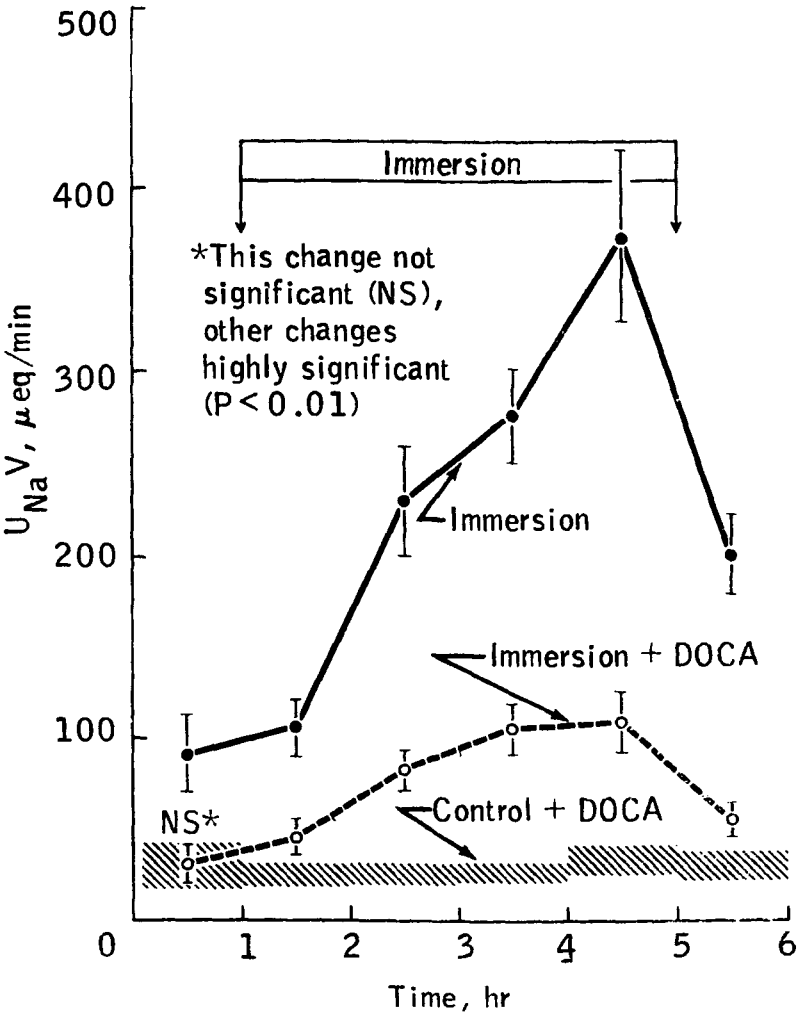


Figure 9-4.- Effect of DOCA pretreatment on renal sodium management during water immersion. Shaded area represents mean ± standard error for control studies (control + DOCA); brackets represent mean ± standard error for immersion and immersion + DOCA. Sodium intake was 150 meq, N = 6.

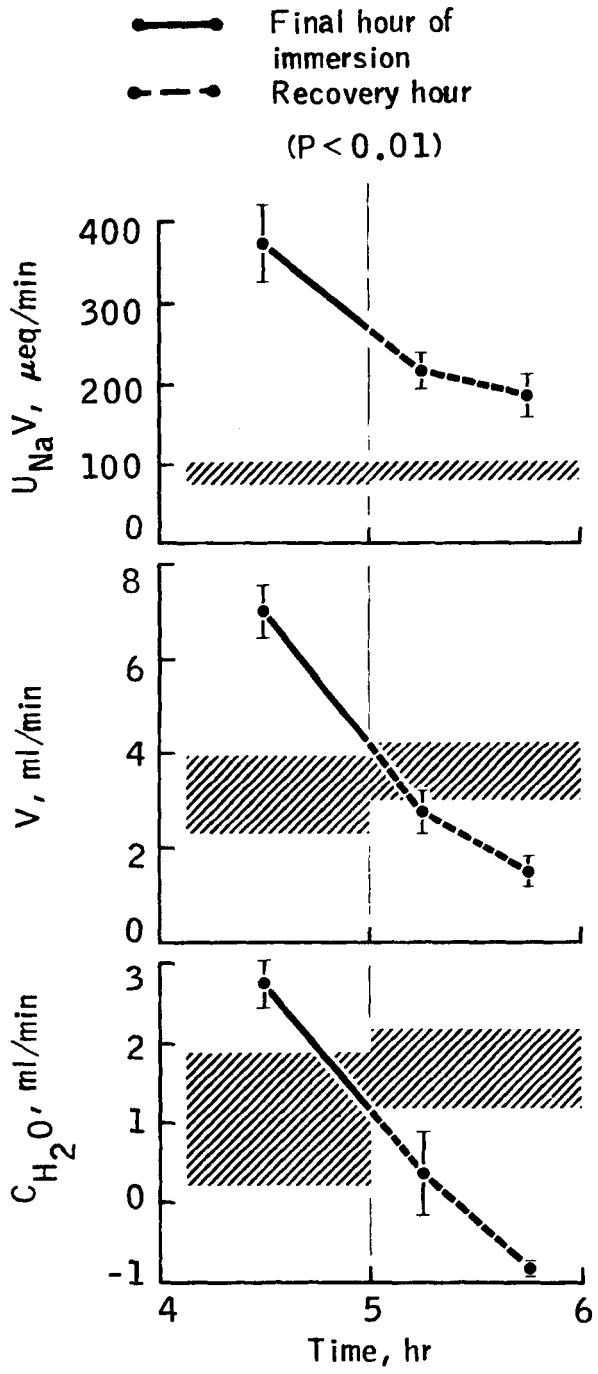


Figure 9-5.- Effect of cessation of water immersion on the rates of sodium excretion ($U_{Na}V$), urine flow (V), and free water clearance (C_{H_2O}). Shaded areas represent mean ± standard error for control hours; brackets represent mean ± standard error for immersion group (from ref. 9).